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LAURA CID FLORES DOS SANTOS

APLICABILIDADE DAS SONDAS FLUORESCENTES E DA AUTOFLUORESCÊNCIA NA DETECÇÃO PRECOCE DE DESORDENS ORAIS POTENCIALMENTE MALIGNAS: UMA REVISÃO SISTEMÁTICA E META-ANÁLISE

Porto Alegre
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Dissertação apresentada como requisito parcial para a obtenção do título de Mestre em Odontologia, área de concentração Estomatologia, ao Programa de Pós-Graduação em Odontologia da Faculdade de Odontologia da Universidade Federal do Rio Grande do Sul.

Orientador: Prof. Dr. Marcelo Lazzaron Lamers

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RESUMO

As desordens orais potencialmente malignas (DOPM) representam um grupo diverso de lesões orais que podem favorecer o desenvolvimento de carcinoma espinocelular oral (CECO). No entanto, as alterações teciduais provocadas pelas DOPM podem ser de difícil detecção clínica para a grande maioria dos profissionais, já que podem ser bastante sutis e ainda podem mimetizar alterações inflamatórias bastante comuns. Diante deste contexto, diversas ferramentas auxiliares estão sendo avaliadas quanto ao seu potencial uso na rotina clínica, podendo facilitar a detecção de tais lesões de maneira precoce. Assim sendo, o objetivo do presente trabalho foi realizar uma revisão sistemática e meta-análise acerca do uso da autofluorescência e de sondas fluorescentes na detecção precoce de desordens orais potencialmente malignas. As bases de dados PubMed, Embase, Scopus, Web of Science foram utilizadas como fonte de busca, assim como as bases Proquest, Open Grey e Google Scholar na literatura cinza. Os estudos incluídos foram aqueles que utilizaram pelo menos um método auxiliar e seguiram os critérios de inclusão estabelecidos. O presente estudo foi registrado no PROSPERO sob o número: CRD42020187911. Ao total, 2.715 artigos foram incluídos e, após as diferentes etapas de seleção, 25 artigos atenderam totalmente os critérios de inclusão. O VELscope® foi o equipamento mais utilizado para autofluorescência, enquanto o ácido aminolevulínico (5-ALA) foi o principal representante das sondas fluorescentes. A metanálise avaliou todos os 10 artigos que utilizaram o VELscope® como método auxiliar durante a detecção de lesões. A sensibilidade combinada foi de 74% (IC95 60-76%, p = 0,0001, havendo diferença significativa entre os grupos) e a especificidade foi de 57% (IC95 52-60%, p = 0,0000, havendo diferença significativa entre os grupos). A inclusão de métodos auxiliares é promissora e tem potencial para auxiliar o cirurgião-dentista na detecção precoce de tais lesões, permitindo que o paciente receba o correto tratamento e possibilitando um melhor prognóstico.

Palavras-chave: Detecção precoce; Autofluorescência; Sondas fluorescentes; 5-ALA; VELscope; Desordens orais potencialmente malignas.

ABSTRACT

Oral potentially malignant disorders (OPMD) represent a diverse group of oral lesions that favor the development of oral squamous cell carcinoma (OSCC). However, tissue changes caused by OPMD may be difficult to detect for the vast majority of professionals, as they can be quite subtle and can even mimic very common inflammatory changes. In this context, several auxiliary tools are being evaluated regarding their potential and use in clinical routine, which may facilitate the early detection of such lesions. Therefore, the objective of the present work was to perform a systematic review and meta-analysis on the use of autofluorescence and fluorescent probes in the early detection of potentially malignant oral disorders. PubMed, Embase, Scopus, Web of Science databases were used as search sources, as well as Proquest, Open Gray and Google Scholar in the gray literature. The included studies were those that used at least one auxiliary method and followed the established inclusion criteria. The present study was registered with PROSPERO under the number: CRD42020187911. In total, 2,715 articles were included, and, after the different selection steps, 25 articles fully met the inclusion criteria. The VELscope® was the most used equipment for autofluorescence, while aminolevulinic acid (5-ALA) was the main representative of the fluorescent probes. The meta-analysis evaluated all 10 articles that used VELscope® as an auxiliary method during lesion detection. The pooled sensitivity was 74% (95CI 60-76%, p=0.0001, with a significant difference between the groups). and the specificity was 57% (CI95 52-60%, p=0.0000, with a significant difference between the groups). The inclusion of auxiliary methods is promising and has the potential to help the dentist in the early detection of such lesions, allowing the patient to receive the correct treatment and enabling a better prognosis.

Keywords: Early detection; Autofluorescence; Fluorescent probes; 5-ALA; VELscope®; Oral potentially malignant disorders.

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OPMD – Oral potentially malignant disorders

5-ALA – Aminolevulinic acid

WHO – World Health Organization

PVL – Proliferative verrucous leukoplakia

OSF – Oral submucous fibrosis

OLP – Oral lichen planus

OLL – Oral lichenoid lesions

AK/AC – Actinic keratosis/Actinic cheilitis

OLE – Oral lupus erythematosus

DC – Dyskeratosis congenita

OGVHD – Oral graft host disease

PPIX – Protoporphyrin

Antecedentes e justificativa

DOPM – Desordens orais potencialmente malignas

LO – Leucoplasia oral

OMS – Organização Mundial da Saúde

CECO – Carcinoma espinocelular oral

LVP – Leucoplasia verrucosa proliferativa

EO – Eritroplasia oral

LP – Líquen plano

LL – Lesões liquenoides

DC – Disceratose congênita

LE – Lúpus eritematoso oral

NADH – Dinucleotídeo de nicotinamida e adenina

FAD – Dinucleotídeo de flavina e adenina

VELscope® - Visually Enhaced Light Scope

5-ALA – Ácido aminolevulínico

SUMÁRIO

1 ANTECEDENTES E JUSTIFICATIVA

1.1 Desordens orais potencialmente malignas

As desordens orais potencialmente malignas (DOPM) correspondem a um distinto grupo de lesões com risco aumentado de malignidade. Leucoplasia, leucoplasia verrucosa proliferativa, eritroplasia, fibrose submucosa oral, lesões liquenoides, queilite actínica, lesões palatinas em fumantes reversos, lúpus eritematoso, disceratose congênita e doença do enxerto oral *versus* hospedeiro referem-se a este grupo (VAIL; ROBINSON; CONDON, 2020; WARNAKULASURIYA; KUJAN; AGUIRRE-URIZAR; BAGAN *et al.*, 2020). Abrangem uma ampla gama de características clínicas, que incluem variações de cor, extensão e alterações topográficas (placa, atrófica, granular, verrucosa) (SPEIGHT; KHURRAM; KUJAN, 2018; WILLIAMS; POH; HOVAN; NG *et al.*, 2008). Grande parte das DOPM podem não progredir para um carcinoma, contudo, fornecem um local de anormalidade que torna o desenvolvimento do câncer mais provável (WARNAKULASURIYA; KUJAN; AGUIRRE-URIZAR; BAGAN *et al.*, 2020).

Além de possibilitar a diminuição das taxas de mortalidade e morbidade, a detecção precoce de DOPM reduziria, drasticamente, o gasto em saúde relacionado as neoplasias malignas da cavidade oral, já que o diagnóstico tardio acarreta um tratamento mais complexo e, consequentemente, de maior custo (MENZIN; LINES; MANNING, 2007). No Brasil, um importante estudo realizado, a partir de levantamento de dados entre 2008 e 2016, revelou que os procedimentos ambulatoriais foram responsáveis pelo maior custo total; entretanto, os procedimentos de internação tiveram um custo por procedimento individual mais elevado. Os valores são substanciais e foram descritos após a correção pela inflação do ano de 2018 (1$ = R$ 2,044): os gastos em saúde relacionados ao câncer oral foram de $495,6 milhões, sendo $251,6 milhões na esfera ambulatorial e $244,0 milhões, hospitalar. Os números ressaltam ainda mais a importância por estratégias mais assertivas no âmbito da prevenção, com foco em fatores de risco e detecção precoce de DOPM e câncer em estágios iniciais, permitindo que o quadro atual possa ser revertido: doença avançada

como diagnóstico mais frequente e consequente pior prognóstico (MILANI; ZARA; DA SILVA; CARDOSO *et al.*, 2021).

Leucoplasia

De acordo com a definição mais recente da Organização Mundial da Saúde (OMS), o termo ‘leucoplasia’ refere-se a "placa branca de risco questionável, tendo excluído outras doenças ou distúrbios conhecidos que não apresentem risco aumentado para câncer” (WARNAKULASURIYA; JOHNSON; VAN DER WAAL, 2007). Ocorrendo predominantemente entre a quinta e sétima décadas de vida (BOUQUOT; GORLIN, 1986), a leucoplasia oral pode ser até seis vezes mais comum em indivíduos tabagistas e/ou etilistas (HOLMSTRUP; VEDTOFTE; REIBEL; STOLTZE, 2006; MASEREJIAN; JOSHIPURA; ROSNER; GIOVANNUCCI et al., 2006).

Clinicamente, as leucoplasias podem ser classificadas em: homogêneas e não-homogêneas (abrangem um componente eritroplásico). Aquelas pertencentes ao primeiro grupo, manifestam-se como lesões brancas, semelhantes a placas, com superfície plana ou enrugada. Já as não-homogêneas, podem apresentar padrão de superfície verrucosa ou nodular (SPEIGHT; KHURRAM; KUJAN, 2018). Apesar de inúmeras opções serem propostas para o tratamento das leucoplasias, ainda não há um consenso sobre o melhor manejo destas lesões, já que ainda não se tem um tratamento capaz de prevenir, de maneira eficaz, recorrências ou o possível desenvolvimento de carcinoma espinocelular (CECO) (LODI; PORTER, 2008). No entanto, após estabelecido o diagnóstico clínico de LO, a biópsia é mandatória para avaliar alterações teciduais presentes e descartar a possibilidade de CECO (AMAGASA; YAMASHIRO; UZAWA, 2011; VAN DER WAAL; SCHEPMAN; VAN DER MEIJ; SMEELE, 1997).

O exame histopatológico pode revelar áreas de hiperceratose, presença de displasia epitelial e até mesmo um carcinoma in situ ou invasivo e o manejo torna-se bastante variável, dependendo do grau de severidade (VILLA; WOO, 2017). Lesões brancas com diagnóstico histopatológico de displasia ou carcinoma in situ devem ser submetidas à excisão cirúrgica com margens seguras (LODI; PORTER, 2008). Alguns estudos demonstram que lesões com

displasia leve podem regredir ao longo do tempo, como também podem representar atipia epitelial reativa de trauma, contribuindo para possível regressão e recorrência (FARAH; WOO; ZAIN; SKLAVOUNOU *et al.*, 2014).

Assim como ocorre para as demais DOPM, o grau de displasia epitelial representa um importante parâmetro para predizer a transformação maligna da LO em CECO (EVREN; BROUNS; WILS; POELL *et al.*, 2020; JAYASOORIYA; DAYARATNE; DISSANAYAKE; WARNAKULASURIYA, 2020). As lesões displásicas apresentam taxa de transformação maligna variando de 7 a 34%, enquanto as lesões não displásicas exibem taxa de progressão entre 0,1 a 14% (LI; ALMAZROOA; CARVO; SALCINES *et al.*, 2021).

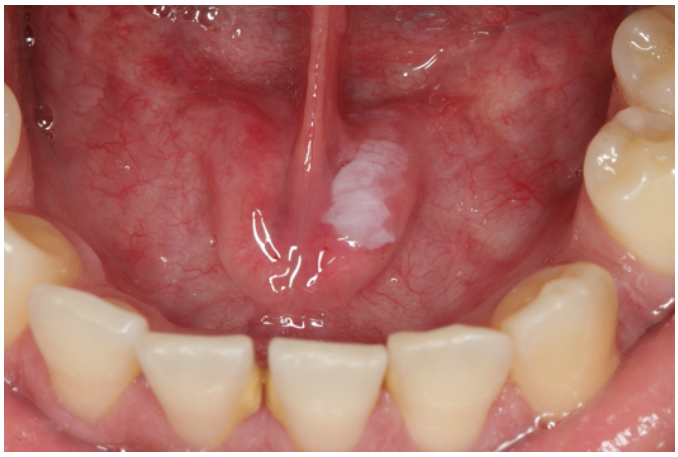

Imagem 1 – Lesão leucoplásica homogênea em assoalho de língua.

Imagem gentilmente cedida pela Profª. Dra. Manoela Domingues Martins (UFRGS).

Leucoplasia verrucosa proliferativa

Reconhecida em 2005 pela Organização Mundial da Saúde (OMS), a leucoplasia verrucosa proliferativa (LVP) foi considerada um subtipo incomum da leucoplasia oral verdadeira (WARNAKULASURIYA; KUJAN; AGUIRRE-URIZAR; BAGAN *et al.*, 2020). Acometendo, preferencialmente, mulheres acima de 60 anos sem associação com consumo de bebidas alcoólicas e/ou tabaco (BAGAN; JIMENEZ; SANCHIS; POVEDA *et al.*, 2003; HANSEN; OLSON; SILVERMAN, 1985), a LVP apresenta comportamento agressivo com elevadas taxas de morbidade e mortalidade, devido ao significativo potencial para transformação maligna (CAMPISI; GIOVANNELLI; AMMATUNA; CAPRA *et al.*, 2004).

Clinicamente, a LVP pode apresentar-se como uma hiperceratose focal, em estágios iniciais, podendo ser facilmente confundida com a leucoplasia convencional (GILLENWATER; VIGNESWARAN; FATANI; SAINTIGNY *et al.*, 2013). As lesões do tipo LVP costumam progredir em 4 distintas fases clínicas: 1) lesão focal precoce; 2) expansão geográfica com o tempo; 3) desenvolvimento de aspecto verrucoide; 4) desenvolvimento de câncer (GILLENWATER; VIGNESWARAN; FATANI; SAINTIGNY *et al.*, 2013). Critérios diagnósticos específicos têm sido propostos e incluem a condição afetando mais de dois sítios orais diferentes e a existência de pelo menos uma área verrucosa (CARRARD; BROUNS; VAN DER WAAL, 2013; CERERO-LAPIEDRA; BALADÉ-MARTÍNEZ; MORENO-LÓPEZ; ESPARZA-GÓMEZ *et al.*, 2010).

Os achados microscópicos da LVP apresentam grande variabilidade, podendo representar desde uma acantose e hiperceratose com presença de infiltrado linfocitário (CAPELLA; GONÇALVES; ABRANTES; GRANDO *et al.*, 2017) ou hiperplasia com ou sem displasia, principalmente nas lesões mais severas de superfície rugosa (MORTON; CABAY; EPSTEIN, 2007). A presença de uma frente invasiva abaixo da mucosa normal adjacente é sugestiva de progressão para carcinoma verrucoso, mesmo que, mais frequentemente, as lesões progridam para CECO (SPEIGHT; KHURRAM; KUJAN, 2018).

O tempo médio para transformação maligna é estimado em 5 a 6 anos após o início das manifestações orais, exigindo o acompanhamento próximo ao paciente e a realização de biópsias frequentes para garantir o diagnóstico precoce e tratamento adequado (SPEIGHT; KHURRAM; KUJAN, 2018).

Eritroplasia

A eritroplasia é definida como “uma mancha vermelha que não pode ser caracterizada clínica ou patologicamente como qualquer outra doença definível de origem traumática, vascular ou inflamatória” (BOUQUOT, 1994; WARNAKULASURIYA, 2018). Ocorrendo predominantemente em homens entre 50 e 70 anos (BOUQUOT; GORLIN, 1986), a eritroplasia oral (EO) representa uma importante lesão precursora de CECO, apresentando taxas de transformação maligna variando de 14 a 50% (REICHART; PHILIPSEN, 2005). A etiologia e a patogênese seguem sendo objeto de estudo; entretanto, alguns fatores predisponentes, como o uso de tabaco e álcool, encontram-se envolvidos

na maioria dos casos (HASHIBE; MATHEW; KURUVILLA; THOMAS *et al.*, 2000).

A biópsia das áreas suspeitas contribui para o diagnóstico definitivo, já que condições inflamatórias podem apresentar características clínicas semelhantes (VAN DER WAAL; SCULLY, 2011; WARNAKULASURIYA, 2019). Lesões eritroplásicas únicas auxiliam o profissional a distinguir de outras condições, como o líquen plano erosivo, lúpus eritematoso e candidíase eritematosa que, frequentemente, se apresentam difundidas em diferentes áreas da cavidade oral (VAN DER WAAL, 2010). No momento do diagnóstico, grande parte das eritroplasias representam, histopatologicamente, um carcinoma ou displasia epitelial moderada/severa (WARNAKULASURIYA; KUJAN; AGUIRRE-URIZAR; BAGAN *et al.*, 2020).

Fibrose submucosa oral

A fibrose submucosa oral é caracterizada como um distúrbio metabólico do colágeno, de caráter debilitante, progressivo e irreversível causado pela mastigação crônica de noz de areca e suas derivações comerciais; acometendo, principalmente, a mucosa oral e, menos frequentemente, a faringe e esôfago (MORE; RAO, 2019). Apresenta distribuição geográfica específica, em função do hábito de mascar o principal agente etiológico (noz de areca), e afeta, predominantemente, indivíduos de origem asiática com igual predileção entre homens e mulheres (PINDBORG; CHAWLA; SRIVASTAVA; GUPTA et al., 1964).

São diversas as alterações sofridas pela mucosa oral quando acometida pela condição, tais como inflamação, ulcerações, pigmentação, perda de resiliência e flexibilidade, tornando-se um tecido de coloração esbranquiçado e fibrosado, levando à rigidez e incapacidade progressiva em abrir a boca. Em estágios mais avançados, hipomobilidade do palato mole e língua, xerostomia, perda das sensações gustativas, distrofia muscular, rouquidão e outros sintomas são frequentemente encontrados (MORE; RAO, 2019; RAJALALITHA; VALI, 2005; WARNAKULASURIYA; JOHNSON; VAN DER WAAL, 2007). Por acometer, de maneira conjunta, músculos, ossos e articulação temporomandibular, a fibrose submucosa oral tem, como significativa

consequência, a limitação da abertura bucal, acarretando dificuldade para realização da higiene oral, fala e alimentação. Até o momento, não se estabeleceu um protocolo de tratamento, mas as opções existentes se subdividem em: tratamento medicamentoso, exercício para melhora da abertura bucal e cirurgia (SHEN; SHIH; FUH; SHIEH, 2020).

As taxas de transformação maligna da fibrose submucosa oral variam de acordo com a localização geográfica. No Brasil, ainda não existem relatos de pacientes acometidos por tal condição. As maiores taxas encontram-se em países asiáticos, variando de 7 a 30% (PENG; LI; CHEN; WANG *et al.*, 2020).
Os achados histológicos compreendem a atrofia epitelial e presença de inflamação crônica, inespecífica, difusa e com áreas de fibrose. Outros sinais podem ser encontrados, tais como reações liquenoides, ulceração da superfície e hiperplasia pseudoepiteliomatosa. A displasia pode ou não ser observada (ISAAC; ISSAC; AHMED KHOSO, 2008).

Líquen plano

O líquen plano (LP) é uma doença autoimune que afeta tanto a pele quanto as membranas mucosas (LAVANYA; JAYANTHI; RAO; RANGANATHAN, 2011). Acomete, predominantemente, mulheres entre a terceira e a sétima décadas de vida. Entende-se que a etiologia do LP representa um processo multifatorial e, fatores relacionados ao sistema imunológico, tais como estresse e ansiedade, podem modular a condição (TORRENTE-CASTELLS; FIGUEIREDO; BERINI-AYTÉS; GAY-ESCODA, 2010; VINCENT; FOTOS; BAKER; WILLIAMS, 1990). Quando em cavidade oral, o LP é visto com frequência em todas as regiões da mucosa, principalmente na mucosa jugal, gengiva e língua (KRUPAA; SANKARI; MASTHAN; RAJESH, 2015). Clinicamente, pode assumir os seguintes subtipos: 1) reticular; 2) atrófico; 3) papular; 4) bolhoso; 5) placa; 5) erosivo ou 6) ulcerativo (INGAFOU; LEAO; PORTER; SCULLY, 2006).

Alguns sinais podem contribuir para o diagnóstico clínico de LP, não descartando a necessidade do exame histopatológico para o estabelecimento de diagnóstico definitivo (ROTARU; SOFINETI; BOLBOACĂ; BULBOACĂ, 2020). Comumente, as lesões orais são bilaterais e, geralmente, simétricas. A

apresentação unilateral é incomum, assim como o acometimento do palato, lábio e assoalho de boca (SCHLOSSER, 2010). As características histológicas do LP compreendem a degeneração hidrópica de células epiteliais basais (queratinócitos), presença de infiltração linfocítica em bandas, podendo haver hiperplasia epitelial, papilas alongadas e/ou afiladas (KHAN; FARAH; SAVAGE; WALSH *et al.*, 2003; SHEN; LIU; ZHU; FENG *et al.*, 2012).

Diferentes modalidades terapêuticas, na presença de sintomatologia, já foram descritas até o momento, sendo as mais comuns a terapia medicamentosa com corticosteroides, imunossupressores e imunomoduladores e também o uso de laser (GUPTA; JAWANDA, 2015).

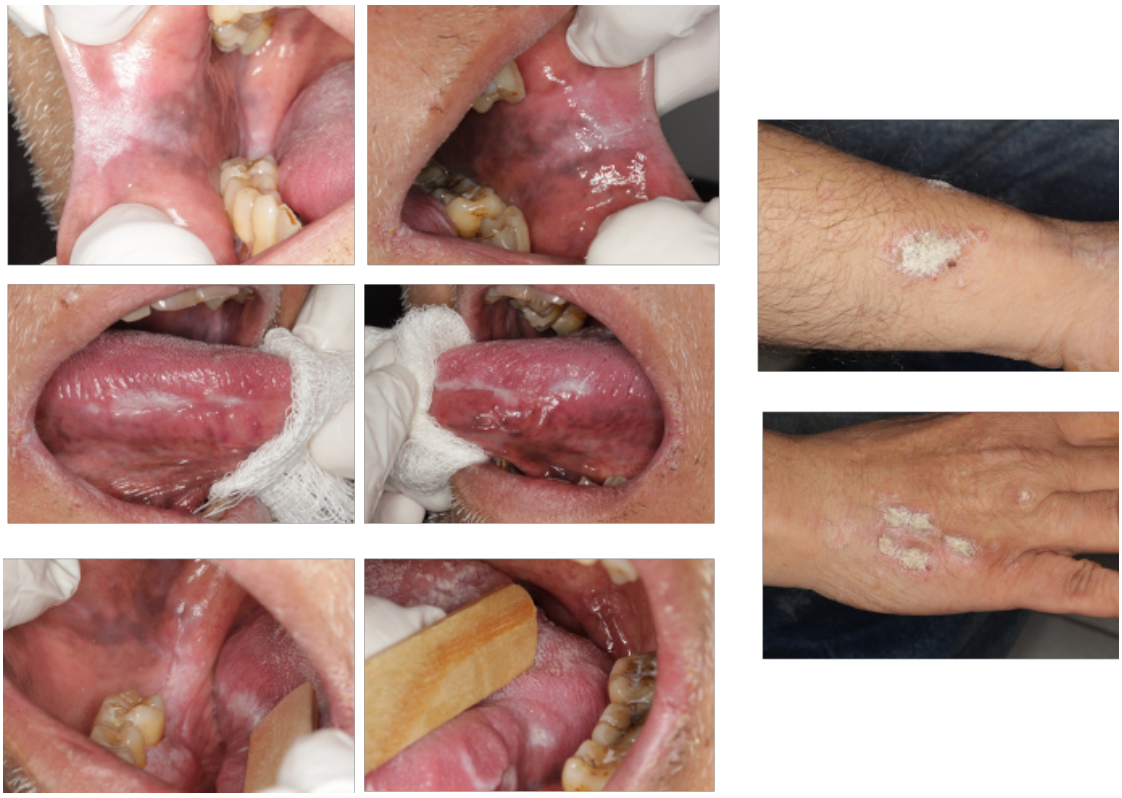

Imagem 2 - Lesões de líquen plano, em um mesmo paciente, acometendo a mucosa bucal e pele.

Imagens cedidas pela Profª. Dra. Manoela Domingues Martins, obtidas durante da Campanha Maio Vermelho, Porto Alegre/RS, Brasil (2015).

Lesões liquenoides

As lesões liquenoides (LL) compreendem aquelas que apresentam características clínicas e/ou histológicas compatíveis com o líquen plano (LP),

podendo estas se apresentar de maneira simétrica e unilateral (WARNAKULASURIYA; KUJAN; AGUIRRE-URIZAR; BAGAN *et al.*, 2020). Na maioria dos casos, as LL se desenvolvem em áreas de contato direto com materiais restauradores, frequentemente, o amálgama e tendem a regredir quando o fator predisponente é identificado e removido (AL-HASHIMI; SCHIFTER; LOCKHART; WRAY *et al.*, 2007). Além daquelas topograficamente associadas a restaurações de amálgama, outros 3 subtipos são descritos: 1) lesões liquenoides relacionadas a drogas; b) lesões liquenoides na presença da doença crônica do enxerto oral *versus* hospedeiro e c) lesões com aspecto semelhante ao líquen plano, mas que carecem de pelo menos uma característica clínica determinante (VAN DER WAAL, 2009).

Clinicamente, as LL podem ser indistinguíveis do LP sendo necessário a realização do exame histopatológico para diferenciação e, principalmente, para avaliar a possível presença de displasia epitelial (VAN DER WAAL, 2009). Os achados microscópicos demonstram um padrão de inflamação perivascular e maior quantidade de células do tipo eosinófilos, plasmócitos e granulócitos (FELDMEYER; SUTER; OESCHGER; CAZZANIGA *et al.*, 2020).

Acredita-se que a taxa de transformação maligna em LL é ainda maior em relação ao LP. Uma meta-análise que avaliou mais de 50 artigos (20.095 pacientes) demonstrou taxa de 1,1% para LP e 2,5% para LL (AGHBARI; ABUSHOUK; ATTIA; ELMARAEZY *et al.*, 2017).

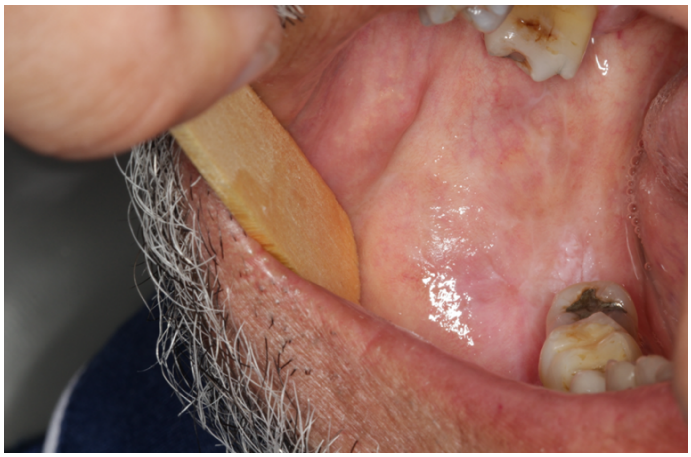

Imagem 3 – Lesão liquenoide próxima a elemento dentário com material restaurador do tipo amálgama.

Imagem cedida pela Profª. Dra. Manoela Domingues Martins (FO/UFRGS).

Queilite actínica

A queilite actínica corresponde a uma condição inflamatória crônica, mais comumente em vermelhão do lábio inferior, resultante da exposição excessiva e desprotegida, de forma crônica e ocupacional a radiação ultravioleta (WARNAKULASURIYA, 2018). Indivíduos de pele clara são os mais acometidos, já que a melanina representa uma proteção natural contra os raios UV (PIÑERA-MARQUES; LORENÇO; SILVA; SOTTO *et al.*, 2010). Abrange uma ampla gama de características clínicas, sendo que as apresentações mais comuns incluem a discromia, lesões brancas, perda da nitidez do limite do lábio, crostas, descamação e ressecamento (TRAGER; FARMER; ULRICH; BASSET-SEGUIN *et al.*, 2021). Histologicamente, a queilite actínica pode apresentar uma série de alterações epiteliais, variando de uma displasia leve a CECO invasivo (DE OLIVEIRA RIBEIRO; DA SILVA; MARTINS-FILHO, 2014).

A taxa de transformação maligna é bastante variável, oscilando entre 10 e 30% (DANCYGER; HEARD; HUANG; SULEY *et al.*, 2018; LOPES; SILVA JÚNIOR; LIMA; OLIVEIRA *et al.*, 2015). É descrito, na literatura, que 95% dos CECO que ocorrem no lábio se desenvolvem a partir de uma queilite actínica preexistente e que a taxa de metástase para lesões em lábio é quatro vezes maior do que na sua contraparte periférica (KARIA; MORGAN; RUIZ; SCHMULTS, 2017). Em relação ao tratamento diversas técnicas podem ser empregadas e, dentre elas, a aplicação de laser e a técnica cirúrgica de vermelhectomia estão associadas a resultados mais favoráveis, com menores taxas de recorrência (TRAGER; FARMER; ULRICH; BASSET-SEGUIN *et al.*, 2021).

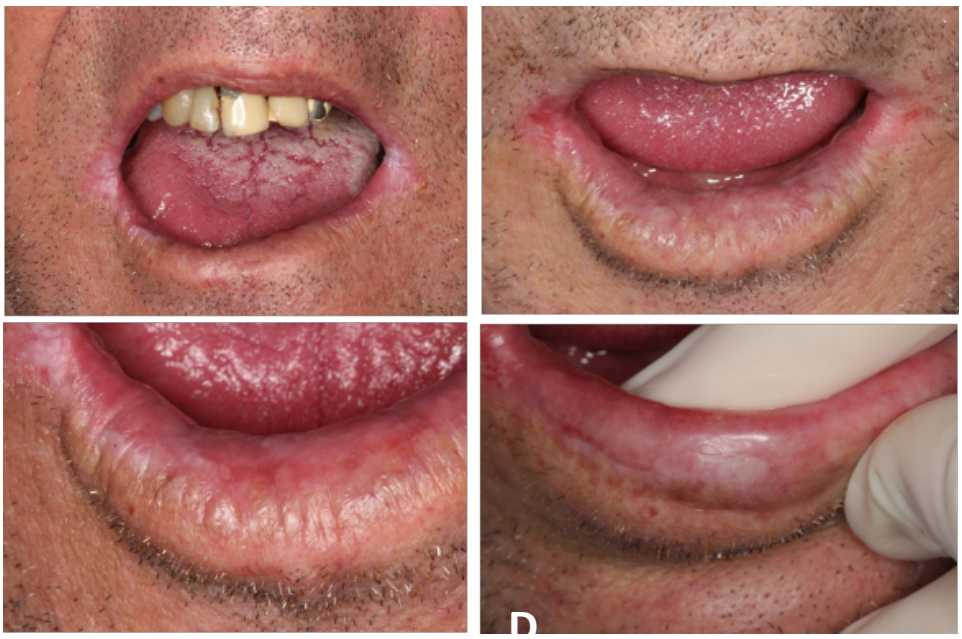

Imagem 4 – Lesões em lábio inferior em indivíduo de pele clara com hábito de exposição excessiva e desprotegida à radiação UV. Presença de discromia, lesões brancas e perda de nitidez entre lábio e pele.

Imagem gentilmente cedida pela Profª. Dra. Manoela Domingues Martins. Acompanhamento do paciente, sem intervenção. Imagens A e B obtidas em 2013 e C e D em 2014.

Lesões palatinas em fumantes reversos

Hábito endêmico comumente praticado em zonas rurais da índia, o tabagismo reverso (RAMULU; RAJU; VENKATARATHNAM; REDDY, 1973) acomete, principalmente, o palato podendo originar lesões leucoplásicas, eritroplásicas e até mesmo leucoeritroplásicas (WARNAKULASURIYA; KUJAN; AGUIRRE-URIZAR; BAGAN *et al.*, 2020). Por provocar o aquecimento do ar no interior da cavidade oral e a temperatura, somada com os subprodutos do tabaco, há um aumento da frequência de lesões orais em comparação com fumantes convencionais (ORTIZ; PIERCE; WILSON, 1996).

Lúpus eritematoso oral

Doença autoimune crônica, o lúpus eritematoso oral (LE) pode ser, clinicamente, semelhante as lesões de líquen plano. Tipicamente, compreende a uma lesão na mucosa oral, circular e atrófica, com ulceração superficial circundada por estriações de cor branca. Os sítios mais comumente afetados

são mucosa bucal, palato e lábios (WARNAKULASURIYA; KUJAN; AGUIRRE-URIZAR; BAGAN *et al.*, 2020).

A literatura descreve que são raros os carcinomas que se desenvolvem a partir de lesões orais de LE, mas a maioria surge na região dos lábios (ARVANITIDOU; NIKITAKIS; GEORGAKI; PAPADOGEORGAKIS *et al.*, 2018).

Disceratose congênita

A disceratose congênita (DC) corresponde a uma doença hereditária rara, caracterizada pela clássica tríade de distrofia ungueal, pigmentação reticular da pele e leucoplasia oral (AULUCK, 2007). Com início dos sintomas entre 5 e 12 anos, a DC acomete, preferencialmente, indivíduos do sexo masculino (SINHA; TRIVEDI; KRISHNA; RAO, 2013). Entretanto, estudos mais recentes demonstram que há grande variabilidade da condição em termos de idade, sintomas e gravidade, o que pode dificultar o estabelecimento de um correto diagnóstico (BEIER; FORONDA; MARTINEZ; BLASCO, 2012).

As lesões leucoplásicas, características da tríade da DC, acometem, com maior frequência, dorso de língua, mucosa bucal e orofaringe (FERNÁNDEZ GARCÍA; TERUYA-FELDSTEIN, 2014). Aproximadamente 30% das lesões evoluem para transformação maligna com desenvolvimento de carcinoma espinocelular entre 10 e 30 anos, tornando necessário o monitoramento frequente destes pacientes e a realização de biópsia das áreas suspeitas (KARUNAKARAN; RAVINDRAN; ARSHAD; RAM *et al.*, 2013).

Doença do enxerto oral versus hospedeiro

A doença do enxerto contra o hospedeiro corresponde uma comum complicação pós-transplante que acomete até metade dos pacientes tratados com células-tronco hematopoiéticas (MAYS; FASSIL; EDWARDS; PAVLETIC *et al.*, 2013). Tem seu início por meio de células T do doador que reconhecem e visam os tecidos do hospedeiro em receptores imunocomprometidos (WARNAKULASURIYA, 2019). A doença do enxerto contra o hospedeiro, mais frequentemente, acomete a pele e cavidade oral; no entanto, diversos tecidos e órgãos podem ser atingidos, como olhos, trato geniturinário, articulações e pulmões (WARNAKULASURIYA, 2019). Quando em cavidade oral, podem ser

observados eritema generalizado da mucosa, erosões, mucoceles, ulcerações, estrias brancas ou pápulas que mimetizam lesões de líquen plano oral (FRICAIN; SIBAUD; HAFIAN; DEMINIÈRE *et al.*, 2005).

Frequentemente, o diagnóstico da condição pode ser obtido a partir das manifestações clínicas. No entanto, a biópsia da mucosa oral e de glândulas salivares menores podem fornecer subsídios importantes para o diagnóstico (GILMAN; SERODY, 2006). Microscopicamente, não existem diferenças significativas entre a forma aguda e crônica. As características encontradas remetem a inflamação da interface liquenoide, exocitose e apoptose de queratinócitos (SHULMAN; KLEINER; LEE; MORTON *et al.*, 2006).

1.2 Dados epidemiológicos

A prevalência mundial de DOPM varia, de acordo com o país de origem e do estilo de vida que podem influenciar no desenvolvimento de determinadas condições (NAPIER; SPEIGHT, 2008), e alcança taxas de 1 a 4% (MELLO; MIGUEL; DUTRA; PORPORATTI *et al.*, 2018).

Em 2011, um importante estudo, visando avaliar a prevalência e indicadores de risco para lesões orais, foi realizado em uma população urbana de Porto Alegre, Rio Grande do Sul. Os resultados demonstraram prevalência de 1,01% para leucoplasias e de 1,02% para lesões de líquen plano (CARRARD; HAAS; RADOS; FILHO *et al.*, 2011).

1.3 Diagnóstico e tratamento

O diagnóstico é baseado na anamnese e exame clínico, sendo necessário a análise histopatológica para confirmação das alterações teciduais (RHODUS; KERR; PATEL, 2014). Dessa maneira, o exame histopatológico é o padrão-ouro para o diagnóstico de DOPM (MELLO; MIGUEL; DUTRA; PORPORATTI *et al.*, 2018). Ainda representando o principal método para o estabelecimento do diagnóstico definitivo, a biópsia, por ser uma técnica invasiva, apresenta algumas limitações técnicas para os profissionais e implicações psicológicas para alguns pacientes. Adicionalmente, quando se tratam de lesões extensas, é

imprescindível a seleção do local mais representativo para análise histológica (MEHROTRA; GUPTA; SINGH; IBRAHIM, 2006).

Por sua vez, o tratamento e manejo destes pacientes são determinados pela avaliação do risco de transformação maligna, tais como fatores clínicos e grau displásico da lesão. As lesões de baixo risco que, histologicamente apresentam displasia de grau leve, podem incluir ao abandono de certos hábitos nocivos e acompanhamento periódico (NADEAU; KERR, 2018).

Quase a totalidade dos carcinomas que acometem a cavidade oral são precedidos por DOPM (NEVILLE; DAY, 2002) e estima-se que aproximadamente 50% dos pacientes com câncer já apresentem metástase locais ou remotas no momento do diagnóstico (YARDIMCI; KUTLUBAY; ENGIN; TUZUN, 2014). A detecção precoce de DOPM ou do câncer em estágios iniciais possibilita a remissão da lesão, atingindo uma taxa de 80% (HADZIC; GOJKOV-VUKELIC; PASIC; DERVISEVIC, 2017). Assim sendo, a correta identificação e caracterização destas lesões é essencial para que o CECO seja abordado, do ponto de vista preventivo, de maneira correta, permitindo a melhora na execução de políticas públicas, taxas de sobrevida e qualidade de vida (AITKEN; VALDIVIA; ADORNO; MATURANA *et al.*, 2017).

1.4 Técnicas auxiliares

Variadas técnicas auxiliares são descritas na literatura com o objetivo de auxiliar a detecção precoce, bem como permitir o acompanhamento próximo de pacientes de alto risco (STEELE; MEYERS, 2011). Dentre as técnicas estão a azul de toluidina, citologia esfoliativa, tecnologias ópticas, espectroscopia Raman, imagem de fluorescência através da autofluorescência tecidual e uso de sondas específicas, imagem de banda estreita e imagem óptica multimodal (HADZIC; GOJKOV-VUKELIC; PASIC; DERVISEVIC, 2017; STEELE; MEYERS, 2011).

Azul de toluidina

O azul de toluidina é um corante do grupo das tiazinas, capaz de se ligar ao DNA e permitir a identificação de ácidos nucleicos presentes nas células

displásicas. Para técnica, se faz necessário a aplicação do corante a 1% sob a mucosa oral e sua remoção é feita logo após, com enxaguante oral com ácido acético a 2% (DRIEMEL; KUNKEL; HULLMANN; VON EGGELING *et al.*, 2007). A intensidade da coloração do corante vai depender do grau de comprometimento epitelial da lesão. Nas lesões benignas, a coloração é mais tênue, quando comparada as displasias epiteliais e carcinomas, onde a marcação do corante é bastante acentuada devido a forte afinidade pela área alterada (KUJAN; GLENNY; OLIVER; THAKKER *et al.*, 2006). Como as hiperplasias benignas, também são capazes de reter o corante, o uso da técnica com azul de toluidina apresenta alta sensibilidade, mas baixa especificidade (PATTON; EPSTEIN; KERR, 2008; SU; YEN; CHIU; CHEN, 2010).

Citologia esfoliativa

A citologia esfoliativa é uma técnica rápida, não-invasiva e indolor, com forte indicação para pacientes com doença sistêmica no qual a biópsia é contraindicada (VERMA; SINGH; BADNI; CHANDRA *et al.*, 2015). Por apresentar tais vantagens, a citologia esfoliativa pode ser realizada vezes para fins de detecção, acompanhamento e pesquisa (RAMAESH; MENDIS; RATNATUNGA; THATTIL, 1999). Em contrapartida, a biópsia e análise histopatológica permanecem como padrão-ouro para o diagnóstico definitivo de lesões orais, restando para a citologia esfoliativa, bem como para demais técnicas, o papel de suporte como um método adjunto (COOKE, 1963).

Seu princípio é baseado na fisiologia epitelial, ou seja, o epitélio normal, quando exposto à esfoliação regular, mantém as células epiteliais fortemente aderidas. Entretanto, a presença de determinadas alterações faz com que as células percam sua capacidade de coesão, resultando na esfoliação (GÖREGEN; AKGÜL; GÜNDOĞDU, 2011). Na prática clínica, o procedimento se inicia após o paciente realizar bochecho para remover possíveis resíduos e, com o *cytobrush*, instrumento próprio para a realização da técnica, o material é coletado. Este é colocado em uma lâmina de vidro, a partir do esfregaço, e após a fixação de mesmo a análise citológica é realizada (GHOM, 2008). Dentro da odontologia, a citologia esfoliativa é mais comumente usada na hipótese de candidíase e leucoplasias pilosas a partir da infecção pelo vírus de Epstein-Barr (WALLING; FLAITZ; ADLER-STORTHZ; NICHOLS, 2003).

Espectroscopia Raman

A espectroscopia Raman, baseada no efeito Raman, é uma técnica de espalhamento de luz que permite a identificação da composição bioquímica de uma determinada amostra, a partir da interação desta com a radiação eletromagnética. Capaz de gerar informações bioquímicas importantes, a espectroscopia Raman reflete a presença e o grau de patologia presente na amostra observada a partir da análise dos principais componentes biológicos, tais como lipídios, proteínas e ácidos nucleicos. (IBRAHIM; TONER; FLINT; BYRNE *et al.*, 2021). As primeiras aplicações em lesões de câncer iniciaram nos anos 2000 e, desde então, diversos estudos têm investigado o potencial uso clínico da espectroscopia Raman (BAKKER SCHUT; WITJES; STERENBORG; SPEELMAN *et al.*, 2000). Esta técnica pode ser utilizada tanto para fins diagnóstico como para a detecção de margens cirúrgicas e para a previsão de resposta ao tratamento (SAHU; KRISHNA, 2017).

1.5 Autofluorescência

Em 1924, a autofluorescência foi descrita pela primeira vez como ferramenta diagnóstica para detecção de alterações da mucosa oral decorrentes do câncer (AWAN; MORGAN; WARNAKULASURIYA, 2011; HANKEN; KRAATZ; SMEETS; HEILAND *et al.*, 2013; POLICARD). Baseia-se no princípio de que fluoróforos existentes no tecido, tais como o dinucleotídeo de nicotinamida e adenina (NADH) e dinucleotídeo de flavina e adenina (FAD), quando excitados por uma fonte de luz externa no comprimento de onda entre 400-460nm, emitem uma fluorescência no espectro da cor verde. No entanto, a mucosa alterada é incapaz de emitir fluorescência devido à interrupção na distribuição dos fluoróforos, sendo observada como uma área escura (AWAN; MORGAN; WARNAKULASURIYA, 2011). A perda da fluorescência em tecidos alterados pode ser explicada devido ao fenômeno da angiogênese, já que a expansão progressiva da densidade de microvasos e o consequente aumento do volume sanguíneo, reduz a capacidade de emitir fluorescência, bem como na

decomposição das ligações cruzadas de colágeno (SVISTUN; ALIZADEH-NADERI; EL-NAGGAR; JACOB *et al.*, 2004).

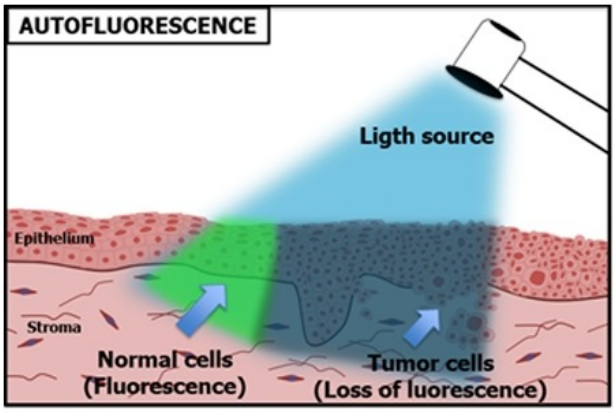

Figura 1 - Princípio de funcionamento da autofluorescência baseado na excitação do tecido, a partir de uma fonte de luz externa, resultando na visualização da fluorescência, de cor verde pálida, em tecidos normais. A presença ou ausência de fluorescência é explicada devido a alteração das concentrações de fluoróforos durante o processo de carcinogênese. Enquanto as células normais emitem fluorescência, as células alteradas serão exibidas por uma área escura, ou seja, perderão a capacidade de fluorescência devido a alteração nas concentrações dos fluoróforos.

Imagem retirada do artigo: *"Use of autofluorescence and fluorescent probes as a potential diagnostic tool for oral cancer: A systematic review"*. Disponível em: https://doi.org/10.1016/j.pdpdt.2020.102073.

O *Visually Enhanced Light Scope* (VELscope®), um dos representantes da técnica, é um dispositivo portátil aprovado em 2006 pela *Food and Drug Administration* (FDA), órgão regulatório dos Estados Unidos, como uma ferramenta adjuvante para aperfeiçoar a visualização de anormalidades da mucosa oral durante o exame clínico convencional (MASCITTI; ORSINI; TOSCO; MONTERUBBIANESI *et al.*, 2018; SHI; LI; SHEN; ZHOU *et al.*, 2019). Ainda no mesmo ano, o primeiro estudo publicado descreveu altos níveis de sensibilidade e especificidade obtidos após a avaliação, do exame clínico e VELscope®, de 44 pacientes com lesões displásica ou CECO confirmadas por biópsia (LANE; GILHULY; WHITEHEAD; ZENG *et al.*, 2006). Atualmente, inúmeros estudos são encontrados na literatura, inclusive aqueles que se concentraram na combinação do VELscope com outro teste diagnóstico para melhorar a detecção de DOPM e CECO (MASCITTI; ORSINI; TOSCO; MONTERUBBIANESI *et al.*, 2018) e, afim de exemplo, pode-se citar a

autofluorescência combinada aos níveis de protoporfirina salivar, que tem se apresentado eficaz na distinção da mucosa normal e anormal (KAUR; JACOBS, 2015).

Recentemente, o dispositivo Evice® (MMOptics, São Carlos, são Paulo, Brasil) desenvolvido no Instituto de Física de São Carlos da Universidade de São Paulo (USP) tem apresentando resultados promissores na detecção de lesões orais. De maneira semelhante ao VELscope®, o Evice® que já obteve a aprovação pela Agência Nacional de Vigilância Sanitária (ANVISA), permite a visualização de fluorescência verde em tecidos saudáveis e vêm sendo utilizado como método auxiliar para detecção de DOPM e como ferramenta de rastreio de pacientes considerados de alto risco (ANDRADE; PRATAVIEIRA; RIBEIRO; BAGNATO *et al.*, 2017).

1.6 Sondas fluorescentes

A principal sonda fluorescente utilizada, com finalidade diagnóstica, é o ácido 5-aminolevulínico (5-ALA) que já comprovou melhorar a eficácia para a detecção precoce do câncer em vários locais do organismo, incluindo a cavidade oral (GILLENWATER; JACOB; GANESHAPPA; KEMP *et al.*, 1998). A técnica é baseada na detecção de alterações teciduais a partir da fluorescência exógena de substâncias que se acumulam no tecido neoplásico (DUNN; DEVINE, 1972; LEONARD; BECK, 1971). O 5-ALA, no entanto, não é capaz de emitir fluorescência, mas provoca o acúmulo seletivo de protoporfirina (PPIX) nos tecidos, resultando em um alto contraste em comparação com o tecido normal circundante (ZHENG; SOO; SIVANANDAN; OLIVO, 2002). A grande vantagem da sonda 5-ALA é que, além de ter a opção de ser aplicado via intravenosa ou tópica, a sua excreção ocorre em menos de 24h, evitando complicações como a fotossensibilização da pele, como é frequente de ocorrer com derivados da hematoporfirina (ZHENG; SOO; SIVANANDAN; OLIVO, 2002).

A fluoresceína, outra sonda amplamente utilizada para a detecção de alterações teciduais, já foi descrita em tumores de cólon, mama, cérebro e estômago (BERGMAN; GLOVICZKI; WELCH; NAESSENS *et al.*, 1992; CAVALLO; DE LAURENTIS; VETRANO; FALCO *et al.*, 2018; VALIVERU;

AGARWAL; AGRAWAL; GAMBHIR *et al.*, 2020). Consolidada na área da medicina oftalmológica, a fluoresceína, um fluoróforo exógeno, pode ser administrada via tópica ou sistêmica, é capaz de emitir uma fluorescência verde-amarelada, possibilitando a detecção e demarcação de diferentes lesões (AG.; M., 2022).

Diante deste contexto, o presente trabalhou buscou avaliar a aplicabilidade clínica da autofluorescência tecidual e das sondas fluorescentes na detecção precoce de desordens orais potencialmente malignas.

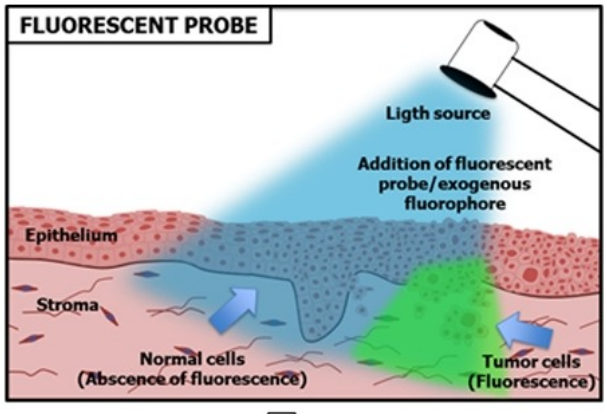

Figura 2 - Princípio de funcionamento das sondas fluorescentes. Diante do tecido alterado, as células acumulam as moléculas no seu interior devido ao elevado metabolismo. A partir de uma fonte de luz externa, as células alteradas emitem fluorescência no espectro verde, enquanto o tecido normal não é capaz de emitir fluorescência.

Imagem retirada do artigo: "Use of autofluorescence and fluorescent probes as a potential diagnostic tool for oral cancer: A systematic review". Disponível em: https://doi.org/10.1016/j.pdpdt.2020.102073.

2 OBJETIVOS

Objetivo geral

Avaliar, através de uma revisão sistemática e metanálise, a eficácia da utilização de métodos adjuntos baseados em autofluorescência e sondas fluorescentes como potencial método de detecção precoce de desordens orais potencialmente malignas.

Objetivos específicos

Realizar busca na literatura sobre uso de autofluorescência e sondas fluorescentes para DOPM;

Avaliar o poder discriminatório da autofluorescência e sondas fluorescentes na detecção precoce de desordens orais potencialmente malignas;

Avaliar quantitativamente o uso de métodos adjuntos baseados em autofluorescências e sondas fluorescentes para detecção precoce de desordens orais potencialmente malignas;

Realizar análise crítica dos protocolos vigentes e seu potencial uso clínico.

3 ARTIGO CIENTÍFICO

Artigo científico publicado na *Photodiagnosis and Photodynamic Therapy* (fator de impacto: 3.631; DOI: 10.1016/j.pdpdt.2022.102764).

A grande maioria das lesões de câncer se desenvolvem a partir de uma DOPM que não foi detectada ao exame clínico. Ao evoluir para um CECO, as taxas de sobrevida do paciente estão diretamente relacionadas ao estágio que se encontra o tumor no momento do diagnóstico. Sendo assim, a detecção precoce de DOPM continua sendo uma importante adversidade dentro da rotina clínica. Diante deste contexto, o presente estudo trata-se de uma revisão sistemática e meta-análise que avaliou a capacidade do mecanismo de autofluorescência e das sondas fluorescentes na detecção precoce de DOPM, sendo utilizado como um mecanismo auxiliar e facilitador do cirurgião-dentista. Observa-se que a inclusão de métodos auxiliares na prática clínica é bastante promissora, tanto para a detecção precoce de DOPM quanto para a avaliação e acompanhamento próximo de pacientes de alto risco.

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Applicability of autofluorescence and fluorescent probes in early detection of oral potentially malignant disorders: A systematic review and meta-data analysis

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ABSTRACT

Oral potentially malignant disorders (OPMD) represent a group of lesions with increased risk for malignant transformation. The management of such injuries is based on surgical treatment or detailed follow-up throughout the patient's lifetime. This systematic review and meta-analysis investigated and critically evaluated the use of autofluorescence and fluorescent probes as potential techniques for the early detection of OPMD. A comprehensive search was performed on Pubmed, Scopus, Web of Science and LIVIVO databases. The gray literature was also consulted and included Google Scholar, Proquest and Open gray databases. 2715 articles were retrieved, and after the different stages of critical evaluation, were reduced to 25 articles that fully met the inclusion criteria. VELscope® was the most used equipment for autofluorescence, while aminolevulinic acid (5-ALA) was the main representative of the probes. The meta-analysis performed included 10 articles that used VELscope® as a method to detect oral disorders. A 95% confidence interval (CI) with a p value significance <0.05 was considered as a criterion for the statistical analysis. The combined sensitivity was 74% (CI95 60–76%, $p = 0.0001$) and the specificity was 57% (CI95 52–60%, $p = 0.0000$). The inclusion of these adjunct methods in clinical practice is very promising, since they are able to help both the clinician and the specialist in the early detection of potentially malignant oral disorders, favoring a better prognosis. However, it is still necessary to carry out further studies, with the aim of establishing a protocol for use and qualification of results.

1. Introduction

Oral potentially malignant disorders (OPMD) refer to a group of lesions and conditions characterized by a variable increased risk for occurrence of cancers of the lip or oral cavity during the lifetime of the patient [1]. According to The World Health Organization (WHO) Collaborating Centre for Oral Cancer, in it is most recent description, leukoplakia, proliferative verrucous leukoplakia (PVL), erythroplakia, oral submucous fibrosis (OSF), oral lichen planus (OLP), oral lichenoid lesions (OLL), actinic keratosis/actinic cheilitis (AK/AC), palatal lesions in reverse smokers, oral lupus erythematosus (OLE), dyskeratosis congenita (DC) and oral graft versus host disease (OGVHD) should be considered OPMD [2]. Among the OPMD, some are commonly encountered such as leukoplakia, OSF, OLP and AC. Oral leukoplakia is the most studied and it is defined as a predominantly white lesion of questionable risk, having excluded (other) known diseases or disorders that carry no increased risk for cancer [1,3]. Leukoplasia is a clinical term, and the histological aspect is correlated with the stage of the

Abbreviations: OPMD, oral potentially malignant disorders; 5-ALA, aminolevulinic acid; WHO, World Health Organization; PVL, proliferative verrucous leukoplakia; OSF, oral submucous fibrosis; OLP, oral lichen planus; OLL, oral lichenoid lesions; AK/AC, actinic keratosis/actinic cheilitis, OLE, oral lupus erythematosus; DC, dyskeratosis congenita; OGVHD, oral graft host disease; PPIX, protoporphyrin.

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lesion, which is characterized by a thickening of the keratin layer (hyperkeratosis), which may or may not be associated with an increase in the spinous layer (acanthosis). Some leukoplakia can even present hyperkeratosis and epithelial atrophy. And epithelial dysplasia, or carcinoma, is found in about 5% to 25% of oral leukoplakia [4]. OPMDs refer to a very heterogeneous group, which may have different clinical characteristics. For this same reason, the literature shows a very varied potential for malignant transformation, with a considerably lower risk in white lesions than in red ones [4–6].

The diagnostic method currently carried out is the conventional oral examination (COE), involving visual inspection and digital palpation, under projected incandescent or halogen illumination, followed by a scalpel biopsy in the places where it is considered suspect. The surgical technique requires precision, requiring the correct selection of the sample, including more than one area, with white and red lesions, if present in the same lesion [7], and must remain intact, adequate size, thickness and depth, without causing damage to the patient [8]. Incisional biopsy followed by histopathological analysis is considered the "gold standard" [9,10], and is essential for the definitive diagnosis [11]. However, the inclusion of auxiliary methods can help professionals in the early detection of oral lesions [12].

Several non-invasive strategies have been introduced to aid in the early detection of malignant or potentially malignant lesions, such as Toluidine coloring, ViziLite and VELscope® [9]. The VELscope® is a portable device that facilitates the visualization of the fluorescence of the oral cavity from the emission of blue light, in the wavelength range from 400 to 460 nm, resulting in the excitation of endogenous fluorophores, such as collagen, elastin, keratin, oxidized flavin adenine dinucleotide (FAD) and nicotine adenine dinucleotide (NADH) [9,13]. The principles of the technique are: 1) scattering of light as it interacts with the tissue, [2] absorption and [3] reflection of light from the tissue surface [14]. In the presence of cellular alterations, there is a change in the concentration of fluorophores, generating an effect on the scattering and absorption of light in the tissue [9]. These techniques based on autofluorescence can be great tool in basic research, as they make it possible to perform measurements in real time on viable samples, without the need for preparative treatments (such as fixation and staining), making them particularly suitable for obtaining information on cell morphology, behavior and energy metabolism, allowing the distinction between normal tissue from neoplastic tissue [15].

Another adjuvant method that has been studied is with fluorescent probes that can aid in lesion detection in real time, with the potential to provide similar sensitivity and specificity for clinical diagnosis of experienced professionals. This technique is relatively simple, fast and accurate, which evaluates the biochemical composition and structure of the tissue fluorescence when exposed to a light source [16]. One of the photosensitizers used id 5-aminolevulinic (5-ALA) acid whose administration can be topical or systemic and it is able to induce protoporphyrin (PPIX), a fluorescent substance. In cases of neoplastic tissue, protoporphyrin accumulates, resulting in high contrast when compared to the normal tissue [17]. In the literature, there is great variability in relation to the sensitivity and specificity of both techniques. However, this occurrence can be explained because it depends on the examiner's interpretation and experience. In addition, the lack of a standardization protocol for the performance of such alternative tests also explains the series of varied results.

The main purpose of this systematic review was to evaluate autofluorescence and fluorescent probes as potential techniques that may be used to facilitate the visualization and management of oral potentially malignant disorders.

2. Materials and methods

2.1. Protocol and registration

This systematic review was performed following the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) statement and the Cochrane guidelines. The systematic review protocol was registered at PROSPERO database CRD42020187911.

2.1.1. Study design

This is a systematic review that aimed to answer the following guiding question PICOS: "Can autofluorescence and/or fluorescent probes (***Intervention***) be used as an accurate tool in the early detection (***Outcome***) of oral potentially malignant disorders (***Population***) when compared to biopsy (***Comparator***) considering only clinical studies (***Study design***)?".

2.1.2. Eligibility criteria

Clinical studies that evaluated tissue autofluorescence and used fluorescent probes as a method of diagnosis and/or treatment of potentially malignant lesions was included.

The exclusion criteria used were: 1) studies not related to the topic; 2) animal studies; 3) in vitro studies; 4) languages other than English; 5) letters to the editor or editorials, conference summaries, personal opinions, meeting abstracts, books and/or book chapters.

2.1.3. Sources of information and search

Two reviewers independently searched the electronic databases. The descriptors were researched in DeCS (Health Sciences Descriptors), MeSH (Medical Subject Headings) and Emtree (Embase Subject Headings) and the boolean operators "AND" and "OR" were used in order to make the search strategy feasable (Table 1). Electronic searches on PubMed, Embase, Scopus, Web of Science and LIVIVO were carried out in May 2020. There was no time limitation during the selection of articles. The results obtained were exported to the EndNote Basic/Online software (Thomson Reuters, Toronto, Canada), desktop version, and the duplicates were removed.

2.1.4. Study selection

The selection of articles was carried out by two blind reviewers (JRF and LCFS) and occurred in three stages. Initially, as a calibration exercise, the reviewers (JRF and LCFS) discussed the eligibility criteria and applied them to a sample of 20% of the retrieved studies to determine the agreement between examiners. After reaching an adequate level of agreement ($\kappa = 0.85$), the reviewers read all the studies independently. A third and fourth reviewer (IFPL and MLL) were responsible for crossing the data and the divergences were resolved by consensus with a senior lecturer in Oral Medicine (MDM).

The primer stage analysis of the titles was made by applying the inclusion criteria and those that were in accordance with the objective of the study were selected for the second stage. In this stage, the analysis of the abstracts was carried out following the same application of inclusion and exclusion criteria. Studies that did not have available abstracts were evaluated in the third stage. The references of all selected articles were analyzed, and 9 articles were included.

2.1.5. Process of data collection and extraction

Two reviewers independently extracted data using specially designed spreadsheets. For each included study, the following data were recorded: author and year, sample (n), age (average), type of sample, comparisons, device used, excitation source, incubation time, fluorescent probe, white light vs. biopsy vs. fluorescence, tissue with higher fluorescence intensity, sensitivity and specificity.

A third reviewer was responsible for crossing the data and the divergences were resolved by consensus.

2.1.6. Critical appraisal of the included studies

The assessment of the methodological quality of the included articles was undertaken by two independent authors (JRF and LCFS) using the "The Joanna Briggs Institute Critical Appraisal tools for use in JBI Systematic Reviews Checklist for Diagnostic Test Accuracy Studies". The

Table 1
Search strategies in the databases.

Databases	Search strategies (August 2021)	Results
PubMed http://www.ncbi.nlm.nih.gov/pubmed	("Fluorescent dyes" OR "Fluorescence Agents" OR "Fluorescent probes" OR "Fluorophore" OR "Fluorochromes") AND ("Precancerous Conditions" OR "Leukoplakia, Oral" OR "Leukoplakia" OR "Leukoplakias" OR "Erythroplasia" OR "Keratosis, Actinic")	96
Scopus https://www.scopus.com/	("Fluorescent dyes" OR "Fluorescence Agents" OR "Fluorescent probes" OR "Fluorophore" OR "Fluorochromes") AND ("Precancerous Conditions" OR "Leukoplakia, Oral" OR "Leukoplakia" OR "Leukoplakias" OR "Erythroplasia" OR "Keratosis, Actinic")	268
Embase https://www.embase.com/	('fluorescent dyes'/exp OR 'fluorescent dyes' OR 'fluorescence agents' OR 'fluorescent probes'/exp OR 'fluorescent probes' OR 'fluorophore'/exp OR 'fluorophore' OR 'fluorochromes') AND ('precancerous conditions'/exp OR 'precancerous conditions' OR 'leukoplakia, oral'/exp OR 'leukoplakia, oral' OR 'leukoplakia'/exp OR 'leukoplakia' OR 'leukoplakias' OR 'erythroplasia'/exp OR 'erythroplasia' OR 'keratosis, actinic'/exp OR 'keratosis, actinic')	279
Web of Science	("Fluorescent dyes" OR "Fluorescence Agents" OR "Fluorescent probes" OR "Fluorophore" OR "Fluorochromes") AND ("Precancerous Conditions" OR "Leukoplakia, Oral" OR "Leukoplakia" OR "Leukoplakias" OR "Erythroplasia" OR "Keratosis, Actinic")	2
LIVIVO	("Fluorescent dyes" OR "Fluorescence Agents" OR "Fluorescent probes" OR "Fluorophore" OR "Fluorochromes") AND ("Precancerous Conditions" OR "Leukoplakia, Oral" OR "Leukoplakia" OR "Leukoplakias" OR "Erythroplasia" OR "Keratosis, Actinic")	299
Google Scholar https://scholar.google.com	("Fluorescent dyes" OR "Fluorescence Agents" OR "Fluorescent probes" OR "Fluorophore" OR "Fluorochromes") AND ("Precancerous Conditions" OR "Leukoplakia, Oral" OR "Leukoplakia" OR "Leukoplakias" OR "Erythroplasia" OR "Keratosis, Actinic")	1250
Open gray http://www.opengrey.eu	("Fluorescent dyes" OR "Fluorescence Agents" OR "Fluorescent probes" OR "Fluorophore" OR "Fluorochromes") AND ("Precancerous Conditions" OR "Leukoplakia, Oral" OR "Leukoplakia" OR "Leukoplakias" OR "Erythroplasia" OR "Keratosis, Actinic")	397
ProQuest https://www.proquest.com	("Fluorescent dyes" OR "Fluorescence Agents" OR "Fluorescent probes" OR "Fluorophore" OR "Fluorochromes") AND ("Precancerous Conditions" OR "Leukoplakia, Oral" OR "Leukoplakia" OR "Leukoplakias" OR "Erythroplasia" OR "Keratosis, Actinic")	124
TOTAL		2715

risk of bias was ranked as ***High*** when the study reached up to 49% of the "yes" score, ***Moderate*** when the study reached from 50% to 69% of the "yes" score, and ***Low*** when the study reached over 70% of the "yes" score (Table 3).

According to the recommendations of the "Joanna Briggs Institute Critical Appraisal Checklist for Diagnostic Test Accuracy Studies", item 5 was considered "Not applicable (NA)" when the diagnostic threshold was not pre-established.

2.1.7. Statistical analysis

The software Meta-analysis of Diagnostic and Screening Tests (MetaDiSc® version 1.4 – http://hrc.es/investigacion/metadisc.html) was employed to integrate and analyze data [18].

The results were presented by plots of sensitivity, specificity, positive likelihood ratio (positive LR), negative likelihood ratio (negative LR) and diagnostic odds ratio (DOR) followed by summary statistics and their confidence intervals (95% CI) of individual studies and corresponding pooled indexes with their confidence intervals (95% CI). All these data were obtained from 2 × 2 tables published in elected studies.

I^2 and Q-tests indicated the degree of inconsistency across the included trails, I^2>50% and p<0.05 indicated heterogeneity. The random effects model (DerSimonian Laird method) was chosen for the heterogeneity analysis.

All analyses adopted in this article were totally based on previous elected studies.

3. Results

3.1. Selection of studies

The search in electronic databases resulted in 2715 articles. A total of 2339 articles were obtained after removing duplicates. Of these, 296 were eligible. After the step of reading the abstracts, 33 articles were analyzed in full. 963 references were verified, and 9 articles were included. Eligibility criteria were evaluated in 42 articles. Of the 42 articles that had their text completely read, 17 were excluded for the following reasons: 1 article written in Russian, 11 articles that did not use such methods for diagnostic purposes, 3 did not mention the definitive diagnosis through histopathological analysis and 2 were developed in animals (Appendix 1). The flowchart depicts the search and the selection process (Fig. 1).

3.2. Study characteristics

Articles from four continents (9 countries) were included. Most cases were reported in Asia (n = 6/41.76%), followed by Europe (n = 6/35.29%), America (n = 3/17.64%) and Oceania, with only one case reported (n = 1/5.88%).

The year of publication of the selected studies ranged from 2000 to 2021. All twenty-five used patients as a sample. The average age ranged from 17 to 82 years. The comparison methods used were oral clinical examination, fluorescence and autofluorescence associated with biopsy (Table 2).

Regarding the outcome, 20 studies evaluated tissue autofluorescence ([12, 19–37]) (80%) and 5 studies used fluorescent probes (20%) [38–42]. Of these, 3 studies used 5-ALA (60%) [39–41] while two used fluorescein (40%) [38,42]. For the administration of the probes, 4 studies used it as topical (80%) [39–42] and one used it intravenously (20%) [38] (Table 2).

In relation to autofluorescence, the device most used to direct the light was the VELscope®, being reported in 12 articles with a percentage 60% [12,19,21–24,26,29–32,34]. Other methods for the same purpose were registered, like a spectroscopic system used in 2 articles (10%) [20,36], a Spectrofluorometer (Fluorolog III) that was reported in 3 articles (15%) [27,28,37] and other Spectrofluorometer was reported in 1 article (5%) [33]. Also, some devices were worn in only one study each, ViziLite (5%) [24], Oralook (5%) [35] and an original device for the detection of the oxidized FAD autofluorescence (5%) [25]. For fluorescents probes the use of an endoscope was reported in 40% [39,40], spectroscopy in 20% [41] and probe-based confocal laser endomicroscopy (pCLE probe) in 20% [38]. One article did not mention the method used (20%) [42].

Three articles reported the analysis of fluorescence images, which was performed using O-SMA-3 optical multichannel analyzer [40], ImageJ software version 1.5 (National Institutes of Health, Bethesda,

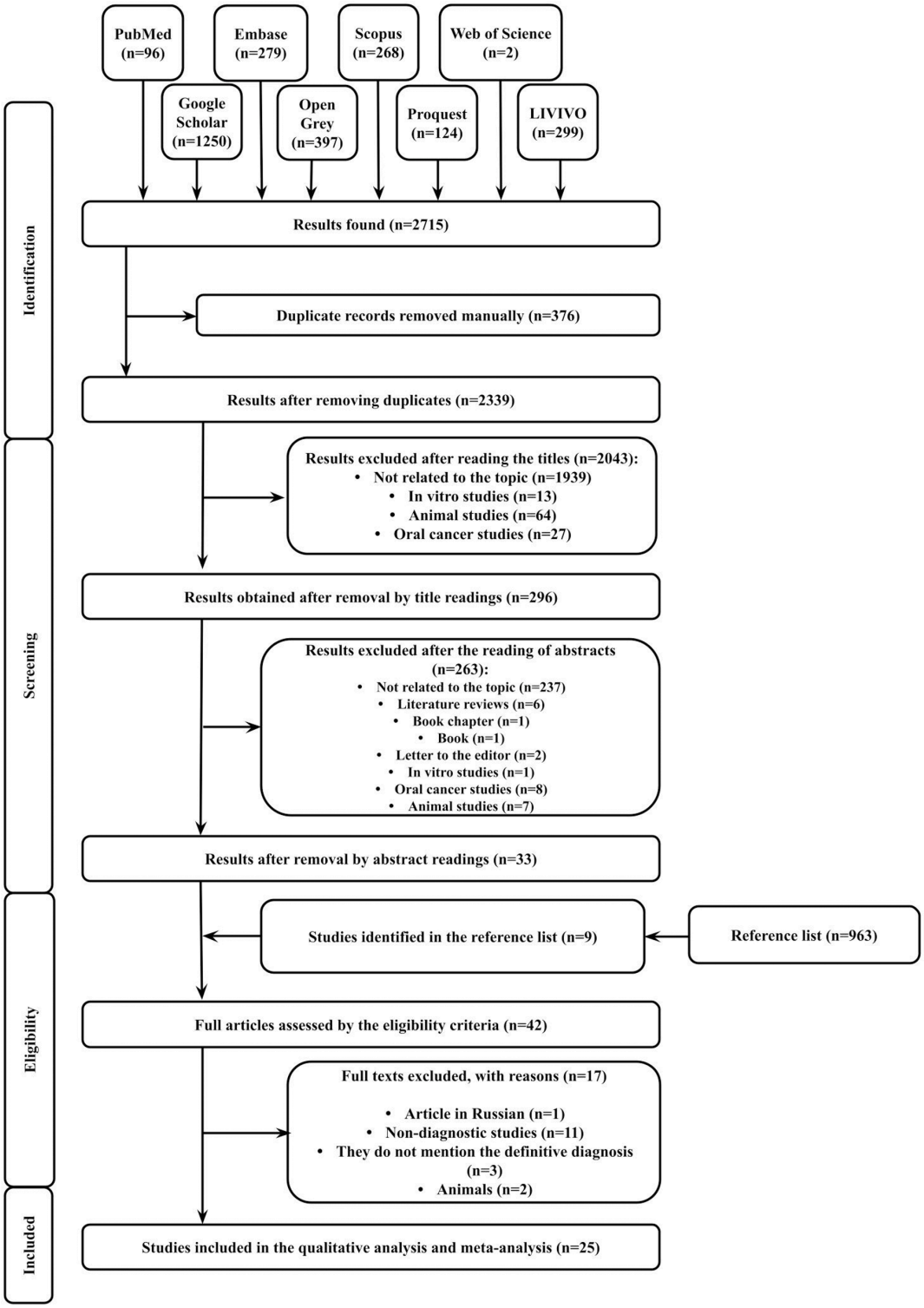

Fig. 1. Flowchart showing the different steps for the selection of articles included in this systematic review and meta-data analysis.

Table 2
Summary of the main characteristics of the eligible studies.

Author, year	Sample	Age and SD (mean)	Comparation	Device	Excitation source	Probe administration	Incubation time	Fluorescent probe (concentration)	Analysis of fluorescence images
Ganga et al., *2017*	200 patients	–	Autofluorescence x Biopsy	VELscope®	–	–	–	Autofluorescence	–
Chaturvedi et al., *2010*	170 patients	50 years	Autofluorescence x Biopsy	Compact and portable spectroscopic system	High-pressure nitrogen laser (375–700 nm)	–	–	Autofluorescence	–
Leunig et al., *2000*	6 patients	53.5 years	–	Endoscope	Xenon short arc lamp blue light (375–440 nm)	Topical	1–2 h	5-ALA (0,4%)	O-SMA-3 optical multichannel analyzer
Amirchaghmaghia et al., *2017*	45 patients	52.3 years SD: 14.8	Autofluorescence x Biopsy	VELscope®	Blue light (400–460 nm)	–	–	Autofluorescence	–
McNamara et al., *2012*	130 patients	45.4 years	Autofluorescence x Biopsy	VELscope®	–	–	–	Autofluorescence	–
Marzouki et al., *2012*	85 patients	61.59 years S. D: 14.2	Autofluorescence X Clinical examination	VELscope®	–	–	–	Autofluorescence	–
Mehrotra et al., *2010*	258 patients (102 patients with ViziLite and 156 with VELscope)	ViziLite: 39 years VELscope: 41 years	Autofluorescence x Biopsy	ViziLite and VELscope®	–	–	–	Autofluorescence	Software (Mathematica 6.0.3, Wolfram Research, Champaign, Ill.)
Moro et al., *2010*	32 patients 12 (affected by potentially malignant disorders)	–	Autofluorescence x Biopsy	Original device for the detection of the oxidized FAD autofluorescence.	Blue violet (450 nm)	–	–	Autofluorescence	
Scheer et al., *2011*	64 patients	59.8 years	Autofluorescence x Biopsy	VELscope®	Blue light (400 - 460 nm)	–	–	Autofluorescence	–
Venugopal et al., *2013*	48 patients 18 (leukoplakia) 30 (normal mucosa)	52.71 years SD: 2.05	Autofluorescence x Biopsy	Spectrofluorometer (Fluorog III)	Xenon lamp (460–750 nm)	–	–	Fluorescence spectroscopy	–
Haris et al., *2009*	31 patients 20 (oral submucous fibrosis) 10 (normal mucosa) 1 (oral submucous fibrosis + oral cancer)	Patients with lesions: 45.43 years SD: 18.09 Normal mucosa: 44.7 years SD: 12.99	–	Fluorolog III	Xenon lamp (450 W)	–	–	Fluorescence spectroscopy	–
Nathan et al., *2014*	21 patients (12 leukoplakia) (9 carcinomas)	64.2 years SD: 15.2	Probe-based confocal laser endomicroscopy images x biopsy	pCLE probe (1.8 mm GastroFlex)	–	Intravenous	15 min	10% fluorescein (2.5 mL)	–
Zheng et al., *2002*	28 patients	58 years	Biopsy x Fluorescence	Endoscope	Xenon lamp (370–450 nm)	Topical	1.5–2 h	5-ALA (0,4%)	–
Paderni et al., *2011*	175 patients	60.38 years SD: 12.26	Biopsy x autofluorescence	VELscope®	Blue-violet light (400–460 nm)	–	–	Autofluorescence	–
Farah et al., *2011*	112 patients	Male 57.8 SD: 11.88 Female 59.08 SD: 12.8	Biopsy x autofluorescence	VELscope®	–	–	–	Autofluorescence	–
Awan et al., *2011*	126 patients	–	Biopsy x autofluorescence	VELscope®	–	–	–	Autofluorescence	–
Elvers et al., *2014*	20 patients 26 lesions	52 years	White light x Fluorescence	VELscope®	Blue light (400 – 460 nm)	–	–	Autofluorescence	–
Chen et al., *2002*	59 patients	38.2 SD: 10.6	Biopsy x autofluorescence	Spectrofluorometer (SkinSkan, JC Inc., Urbana, IL, USA).	–	–	–	Autofluorescence	–
Giovannacci et al., *2021*	60 patients 108 lesions	69 years	Biopsy x autofluorescence	VELscope®	Blue-violet light (410–430 nm)	–	–	Autofluorescence	–
Morikawa et al., *2020*	314 patients	68.3 years	Biopsy x autofluorescence	ORALOOK (Hits Plan Inc., Tokyo, Japan) or		–	–	Autofluorescence	ImageJ software version 1.5

(*continued on next page*)

Table 2 (*continued*)

Author, year	Sample	Age and SD (mean)	Comparation	Device	Excitation source	Probe administration	Incubation time	Fluorescent probe (concentration)	Analysis of fluorescence images
				IllumiScan (Shofu Inc., Kyoto, Japan).	Blue light (400 – 460 nm)				(National Institutes of Health,Bethesda, MD, USA).
Wang et al., *2009*	40 patients	–	Biopsy x fluorescence images	Spectroscopy	–	Topical	90 min	5-ALA (0.05 mL/ cm^2)	–
Tsai et al., *2003*	149 patients with clinical lesions 15 healthy volunteers	–	Biopsy x autofluorescence	Spectroscopy	Xenon lamp (150 W)	–	–	Autofluorescence	–
Qaiser et al., *2020*	42 patients with oral potentially malignant disorders	45.98 SD: 13.93	Biopsy x fluorescence images	–	Blue light (480 nm)	Topical	5 min	Fluorescein	–
Ramesh et al., *2020*	20 patients with oral lichen planus 16 controls	Patients with lesions: 17–69 years Controls: 25–56 years	Biopsy x autofluorescence	Fluorolog III	Xenon lamp (450 W)	–	–	Fluorescence spectroscopy	–
Hanken et al., *2013*	120 patients White light = 60 White light plus VELscope = 60	White light: 38–82 years White light plus VELscope: 41–76 years	Biopsy x autofluorescence	VELscope®	Light in the 430 nm wave	—	–	Autofluorescence	–

SD: Standard deviation;.

MD, USA) [35] and Software (Mathematica 6.0.3, Wolfram Research, Champaign, Ill) [24] (Table 2).

3.3. Risk of bias of studies

Fourteen articles had a low risk of bias (56%) [12,19–21,24,26,29–32,34,35,38,41] while 9 articles had a moderate risk (36%) [22,23,27,28,33,36,37,39,42] and only 2 had a high risk of bias (8%) [25,40] (Table 3).

3.4. Individual results of the studies

In this systematic review, 20 articles evaluated autofluorescence (n = 2249 patients) in which 19 (n = 2229 / 95%) used it as a method of early detection and 1 article used autofluorescence to observe possible signs of dysplasia in the leukoplakia margins (n = 20 / 5%) [30] (Table 2).

Regarding the diagnosis, leukoplakia were the lesions most detected by autofluorescence, although other lesions were also diagnosed, such as OSF, OLP and erythroplakia. When compared to histopathological analysis, 164 dysplastic lesions were diagnosed and classified according to their severity. One article (n = 258 patients) evaluated the effectiveness of ViziLite and VELscope® to diagnose dysplastic lesions, but neither device was able to diagnose lesions that were not suggestive, such as dysplasia, in conventional clinical examination [24]. Sensitivity was 0% for ViziLite and 50% for VELscope® and specificity was 75.5% and 38.9%, respectively [24] (Table 4).

Of the five articles that analyzed the fluorescent probes (n = 137 patients), 3 (n = 74/60%) used the 5-aminolevulinic acid (5-ALA), and one of these (n = 21/33.3%) used as a fluorescence marker for malignant tissue during the treatment of leukoplakia [40]. Zheng et al. [39] (n = 28) mentioned the sensitivity and specificity rates, which were 95% and 97% respectively. Nathan et al. [38], and Qaiser et al. [42], also reported sensitivity values of 80% and 96.6% and specificity of 100% and 52.4%, respectively (Table 5). Regarding the time elapsed to maximum fluorescence intensity, each study reported a different time, Zheng et al. [39], that was 1,5–2 h, Nathan et al. [38], 15 min, Wang et al. [41], 90 min and Qaiser et al. [42], 5 min. Leunig et al. [40], did not provide any of these data (Table 5).

In relation to the outcome, Qaiser et al. [42] evaluated 58 cases of OSCC and dysplasia, in which 56 were positive for fluorescence. The study by Nathan et al. [38], using fluorescent probes, detected 5 out of 7 lesions with signs of dysplasia observed through histopathological examination. Two articles evaluated fluorescence peaks in OPMD looking for signs of epithelial hyperkeratosis and/or epithelial dysplasia [39,41] and Leunig et al. [40] used 5-ALA as a fluorescent marker to assess signs of dysplasia in leukoplastic lesions during treatment with retinyl palmitate.

3.5. Statistical output

The present meta-analysis included 10 articles that evaluated autofluorescence using VELscope® for early detection of potentially malignant oral disorders. Articles that evaluated fluorescent probes and/or measured fluorescence peaks were excluded from the statistical analysis for not presenting the necessary data. Confidence interval (CI) 95% with p value significance taken at < 0.05 was taken as criteria for obtaining the statistical analysis. The results were presented in sensitivity plots (Fig. 2A), specificity (Fig. 2B), positive likelihood ratio (Fig. 3A), negative likelihood ratio (Fig. 3B) and diagnostic odds ratio (Fig. 3C).

The sensitivity, defined as the ability of the test to be positive when there is a disease [43] obtained a pooled result of 74% (CI95 68–80%, p = 0.0001). Regarding the specificity (Fig. 2B), determined as the ability of the test to be negative when there is no disease [43], the values are dispersed in the plot, with most studies to the right of the same. The combined specificity was 57% (CI95 53–60%, p = 0.0000).

The positive likelihood ratio (positive LR), defined as the patient's probability of presenting the disease when the test result is positive [43], was 1.65 when pooled (Fig. 3A). In addition, the negative likelihood ratio (negative LR), that is, the patient's chance of not having the disease when the test result is negative, was pooled in 0.69 (Fig. 3B).

The diagnostic odds ratio (DOR), illustrated in Fig. 3C and defined as a measure of the effectiveness of a diagnostic test [43] evaluated in 8 articles, was 2.55.

4. Discussion

The need for alternative methods capable of early detection of OPMD is increasingly necessary, because in addition to the fact that there is no precise method capable of predicting the cancerization of these lesions,

these devices are able to assist in the monitoring and delineation of surgical margins, during the operation, in an attempt to prevent recurrences [44,45]. The current approach to this group of lesions often includes surgical treatment in an attempt to prevent the development of malignancy or the adoption of strict monitoring throughout the patient's life [46]. Therefore, the use of fluorescence would provide a better visualization of tissue changes, indicating the most critical moment for a surgical intervention. This systematic review presents strengths: a broad and careful search strategy in important databases followed by a complete analysis of the selected articles without restriction of year, publication status or language, in addition to conducting a meta-analysis, enabling the composite results by a high rate of sensitivity and moderate specificity, which justifies the use of this method.

Fluorescent probes have also been used as an auxiliary method in the detection of oral lesions, although few studies are found in the literature. The technique based on ALA fluorescence in head and neck tumors started in 1996 and, since then, it has presented itself as a promising technique in pre and postoperative diagnosis [47]. It is based on the use of an exogenous fluorophore that, when excited, induces tissue fluorescence [48]. Among the most commonly used fluorophores is aminolevulinic acid (5-ALA) which targets neoplastic cells and can be administered intravenously or topically [49]. In 2017, 5-ALA was approved by the US Food and Drug Administration as an adjunct for intraoperative visualization in cases of patients with suspected high-grade gliomas [50], and since then, numerous studies have been conducted for this purpose. In a randomized clinical trial developed by Stummer et al. [51], the use of 5-ALA effectively increased the distinction between normal tissue and neoplastic tissue: 65% of 139 patients had complete glioma resection, while only 36% of 131 patients did conventional light surgery group had the same outcome. This data corroborates the results found in the present work, since the use of fluorescent probes enabled the identification of dysplastic lesions. As it is a relatively new technique, few studies are found in other areas, which can make it difficult to measure its effectiveness beyond the medical area and define future perspectives.

Probe Based Confocal Laser Endomicroscopy system (pCLE) is a method that enables to analyze histology in real-time [52]. This new concept is part of a type of optical imaging technique, which uses a single distal lens and an objective lens, to focus the laser and transfer the light exerted by the tissue, respectively [53]. Table VI presents a comparison between the sensitivity and specificity of this method, Raman spectroscopy and COE. In our review, we observed that, of the 5 articles that used fluorescent probes, only 1 used the method with pCLE and intravenous fluorescein, revealing sensitivity of 80% and specificity of 100% [38]. However, the sample was small. In the literature, the pCLE system has also been used to identify other tumors such as lung [54] and colon cancer [55].

Autofluorescence is a diagnostic method based on the excitation of endogenous fluorophores, such as amino acids, metabolic products and structural proteins, through an extrinsic light source. The fluorophores most relevant to the oral cavity are NADH and FAD, present in the epithelium, and cross-linked collagen in the stroma [56], and when unchanged, they emit pale green fluorescence. The present technique has already been explored in the most diverse medical areas, being used in the management of melanomas [57], gliomas [58] and lung injuries [59]. Thus, the use of autofluorescence can also be promising in the face of oral lesions.

The results of sensitivity and specificity, described in Figs. 2 and 3, varied significantly, which is expected in diagnostic tests [43]. In relation to the sensitivity, most of the articles had a value above 60%. Scheer et al. [26] have the sensitivity value toward the extreme left of the graph, and they concluded that autofluorescence can be used as a method of early detection of potentially malignant oral disorders. The pooled specificity was 57% and, for this reason, caution is required when analyzing the data, as this technique may be more likely to give false-positive results [60]. The discrepancy between studies can be explained due to numerous parameters, such as random error, differences in the fundamentals of the diagnostic methods, generation differences between the equipment, equipment calibration and interobserver differences [43]. In addition, studies that achieved

Table 3
Risk of bias assessed by the Joanna Briggs Institute Critical Appraisal Checklist for Diagnostic Test Accuracy Studies.

Author, year	Q.1	Q.2	Q.3	Q.4	Q.5	Q.6	Q.7	Q.8	Q.9	Q.10	% Yes/Risk
Ganga et al., *2017*	√	√	U	√	NA	√	√	√	√	√	80%(Low)
Chaturvedi et al., *2010*	U	√	√	√	NA	√	NA	√	√	√	70%(Low)
Leunig et al., *2000*	U	–	√	NA	NA	√	NA	U	√	√	40%(High)
Amirchaghmaghi et al., *2017*	√	√	√	√	NA	√	U	√	√	√	80%(Low)
McNamara et al., *2012*	√	√	√	√	NA	√	U	√	–	–	60%(Moderate)
Marzouki et al., *2012*	U	√	U	√	NA	√	√	U	√	√	60%(Moderate)
Mehrotra et al., *2010*	√	√	–	√	NA	√	√	√	√	√	80%(Low)
Moro et al., *2010*	–	√	U	U	NA	√	U	U	–	√	30%(High)
Scheer et al., *2011*	U	√	√	√	NA	√	U	√	√	√	70%(Low)
Venugopal et al., *2013*	√	–	U	√	NA	U	U	√	√	√	50%(Moderate)
Haris et al., *2009*	√	–	√	√	NA	U	U	√	√	√	60%(Moderate)
Nathan et al., *2014*	U	–	√	√	NA	√	√	√	√	√	70%(Low)
Zheng et al., *2002*	U	√	U	√	NA	√	U	√	√	√	60%(Moderate)
Paderni et al., *2011*	√	√	√	√	NA	√	√	√	√	√	90%(Low)
Farah et al., *2011*	√	√	√	√	NA	√	√	√	√	√	90%(Low)
Awan et al., *2011*	√	√	√	√	NA	√	√	√	√	√	80%(Low)
Elvers et al., *2014*	√	√	√	√	NA	√	U	√	√	√	80%(Low)
Chen et al., *2002*	U	√	U	√	NA	√	U	√	√	√	60%(Moderate)
Giovannacci et al., *2021*	U	√	√	√	NA	√	√	√	√	√	80%(Low)
Morikawa et al., *2020*	√	√	√	U	NA	√	U	√	√	√	70%(Low)
Wang et al., *2009*	U	√	√	U	NA	√	√	√	√	√	70%(Low)
Tsai et al., *2003*	U	√	U	U	NA	√	√	√	√	√	60%(Moderate)
Qaiser et al., *2020*	U	–	√	U	NA	√	U	√	√	√	50%(Moderate)
Ramesh et al., *2020*	–	√	√	U	NA	√	U	√	√	√	60%(Moderate)
Hanken et al., *2013*	U	√	√	√	NA	√	√	√	√	√	80%(Low)

Q.1: Was a consecutive or random sample of patients enrolled?; Q.2: Was a case control design avoided?; Q.3: Did the study avoid inappropriate exclusions?; Q.4: Were the index test results interpreted without knowledge of the results of the reference standard?; Q.5: If a threshold was used, was it pre-specified?; Q.6: Is the reference standard likely to correctly classify the target condition?; Q.7: Were the reference standard results interpreted without knowledge of the results of the index test?; Q.8: Was there an appropriate interval between index test and reference standard?; Q.9: Did all patients receive the same reference standard?; Q.10: Were all patients included in the analysis?; √ = Yes; – = No; *U* = Unclear; NA = Not applicable.

Table 4
Summary of the main results of the eligible studies (autofluorescence).

Authors/ Year	Patients diagnosed with potentially malignant disorders/ total sample	Comparation	Tissue with higher fluorescence intensity	Sensitivity	Specificity
Ganga et al., *2017*	Leukoplakia 3/43 Oral submucous fibrosis 0/58 Oral lichen planus 18/22	Autofluorescence was compatible with biopsy in 67,5% of cases	Normal tissue	76%	66.29%
Chaturvedi et al., 2010	Potentially Malignant 150/178	Autofluorescence was compatible with biopsy in 84,26% of cases	Normal tissue	91%	90%
Amirchaghmaghia et., 2017	6/8 Mild dysplasia 2/2 Moderate dysplasia 2/2 Severe dysplasia		Normal tissue	83% for premaligant lesion Total 90%	12% for premaligant lesion Total 15%
McNamara et al., *2012*	Severe epithelial dysplasia 1/1 Moderate epithelial dysplasia 0/1	The results found by VELscope® were incompatible with histology	Normal tissue	–	–
Marzouki et al., *2012*	Group 2 - 16 areas were suspicious on the VELscope® and not on the clinical examination (5 were positive for mild, moderate, or severe dysplasia) Group 3 - 12 areas were suspicious by both methods (7 were positive for malignant and premalignant changes)	The diagnostic yield from the regular examination is 47% and the diagnostic yield with the addition of the VELscope® is an additional 31%	Normal tissue	92%	77%
Mehrotra et al., *2010*	14 patients diagnosed with dysplasia/ 258 patients ViziLite 0/3 VELscope® 5/ 11	Neither ViziLite nor VELscope® identified any lesions that were not already apparent during the clinical examination with conventional light	Normal tissue	ViziLite 0% VELscope® 50%*	ViziLite 75.5% VELscope® 38.9%
Moro et al., *2010*	8/12 patients with potentially malignant disorders 2/8	There is no significant difference in the number of lesions diagnosed by the 2 methods	Normal tissue	100%*	93%*
Scheer et al., *2011*	Moderate dysplasia 2/2 Severe dysplasia 2/2 Lichen planus 4/6	VELscope® identified all patients with dysplasia	Normal tissue	100%*	80.8%*
Venugopal et al., *2013*	Leukoplakia 18	–	Normal tissue	96.77%	100%
Haris et al., *2009*	Submucosal Fibrosis (OSF): 20 cases	Autofluorescence spectroscopy can successfully differentiate precancerous from normal mucosa	Premalignant tissue	–	–
Paderni et al., *2011*	Leukoplakia with no dysplasia 1/50 Leukoplakia with dysplasia 10/16 Oral lichen planus 2/64 Verrucous proliferative leukoplakia with dysplasia 2/3 Verrucous proliferative leukoplakia with no dysplasia 0/3 Leuko-erythroplakia with no dysplasia 0/1 Leuko-erythroplakia with moderate/ severe dysplasia 4/5	The VELscope® device cannot fully replace histopathology procedure, which still represents the gold standard for definite diagnosis of OPMD	Normal tissue	65.5%	97.4%
Farah et al., *2011*	Homogenous leukoplakia/keratosis 30/ 53 Nonhomogenous leukoplakia/ suspicious for malignancy 8/27 Lesions with lichenoid features 27/32	VELscope® seems to be of use at aiding the visualization of potentially malignant conditions	Normal tissue	30%	63%
Awan et al., *2011*	Leuko/erythroplakia 61/70 Dysplasia 37/44	VELscope® detected all severe dysplasia and erythroplakia cases	Normal tissue	87.1% (Leukoplakia or erythroplakia) 84.1% (Oral epithelial dysplasia)	21.4% (Leukoplakia or erythroplakia) 15.3% (Oral epithelial dysplasia)
Elvers et al., *2014*	10/26 Leukoplakia *No specimen showed signs of dysplasia.*	Autofluorescence enables to measure the extent of oral leukoplakia beyond their visible margins	Normal tissue	–	–
Chen et al., *2002*	59 OSF mucosal sites	Autofluorescence appears to be a very viable method for real-time diagnosis of OSF due to its unique pattern of autofluorescence spectrum	Premalignant tissue	–	–
Giovannacci et al., *2021*	Mild/moderate dysplasia 5/29 Severe dysplasia/ in situ carcinoma 11/17	Despite its technical limitations, autofluorescence in conjunction with conventional clinical examination has high sensitivity. Quantitative fluorescence assessment can objectively and more accurately discriminate the various lesions and their histological stage	Normal tissue	81%	76.7%
Morikawa et al., *2020*	Oral lichen planus 87/98 Leukoplakia 11/73	Oral cancer screening using an optical instrument can effectively facilitate the early detection of oral lesions	Normal tissue	98%	43.2%
Tsai et al., *2003*	Dysplasia: 15 Dysplasia with OSF: 12	Autofluorescence spectroscopy is a good technique for diagnosis of epithelial hyperkeratosis, epithelial dysplasia and squamous cell carcinomas	Premalignant tissue	100%	93%
Ramesh et al., *2020*	OLP: 20 patients	Autofluorescence is an efficient tool for the non-invasive diagnosis of oral lichen planus	Premalignant tissue	–	–

(*continued on next page*)

Table 4 (*continued*)

Authors/ Year	Patients diagnosed with potentially malignant disorders/ total sample	Comparation	Tissue with higher fluorescence intensity	Sensitivity	Specificity
Hanken et al., *2013*	Dysplastic lesions: 46/47	The VELscope® device is a simple, non-invasive test of the oral mucosa, which can help the experienced clinician to find oral precursor malignant lesions	Normal tissue	97.9%	41.7%

*The data were presented in general, including malignant lesions.

Table 5
Summary of the main results of the eligible studies (fluorescent probes).

Authors/ Year	Patients diagnosed with potentially malignant disorders / total sample	Comparation	Tissue with higher fluorescence intensity	Time elapsed to maximum fluorescence intensity	Sensitivity	Specificity
Nathan et al., *2014*	12/21	The fluorescence results were compatible with the histopathological findings	Premalignant tissue	15 min	80%	100%
Zheng et al., *2002*	8/70	–	Red: malignant tissue Green: benign tissue	1,5–2 h	95%	97%
Leunig et al., *2000*	3/6	5-ALA-induced PpIX fluorescence can be used for monitoring cases of leukoplakia and improved or disappeared in five of six cases	Premalignant tissue		–	–
Wang et al., *2009*	40 patients	ALA-derived PpIX fluorescence spectroscopy might be useful for early diagnosis of oral premalignant lesions	Premalignant tissue	90 min	–	–
Qaiser et al., *2020*	Cases with OSCC and dysplasia 56/58	Fluorescein staining along with blue light is likely to improve detection of early oral cancers and dysplasia and can play a vital role in mass screening programmes of oral cancer	Premalignant/ malignant tissue	5 min	96.6%	52.4%

sensitivity close to 100% obtained relatively small samples, which makes it possible to increase the diagnostic capacity. Likewise, it is believed that the values of sensitivity and specificity are dependent on the degree of development of the lesion, increasing as it reaches a more significant dysplastic level [61]. The meta-analysis conducted by Chaitanya et al. [62] aimed to evaluate the efficacy of autofluorescence through the sensitivity and specificity of specific devices for it. Most studies showed sensitivity above 70% and sensitivity with great variability, corroborating the results of this meta-analysis and reinforcing that autofluorescence can be useful in detecting dysplastic alterations. Therefore, the values related to false positives are not of significant importance on this occasion, since the biopsy must be performed prior to establishing the definitive treatment. The opposite leads to a disadvantage for the patient, since it would be a false negative.

According to the literature, positive LR values, in general, should be greater than 1. This means that the higher the value obtained, the more likely that the test will be positive in individuals who actually have the disease, considering that the test in question contributes significantly to the diagnosis [63]. The positive likelihood obtained in this systematic review was 1.59. The opposite occurs with negative likelihood, which, when less than 1, the less likely the test is to obtain negative results in individuals with the disease, ruling out the diagnosis [63]. The negative likelihood obtained was 0.77, very close to that described in the study by Kim et al. [60], which had a pooled negative predictive value of 0.79. The diagnostic odds ratio was 2.03 and represents the test's effectiveness. Therefore, the higher the value, the better its discriminatory performance [64]. Among clinical applications, DOR is likely to depend on the spectrum of disease severity, as is the case with other test performance indicators [65,66].

In general, some of the points that justify the use of autofluorescence to detect oral lesions are because it is a relatively simple and non-invasive method, providing results in real time and can still be performed by several operators after brief training. The cost for the implementation of such equipment based on autofluorescence can be considered high, but when compared to the expenditure on cancer care for patients with oral cancer, it becomes minimal. A study by Zavras et al. [67], sought to quantify the direct costs of oral cancer treatment in Greece, and present an average treatment cost of US$7450 per patient. In Netherlands, similar treatment for primary oral cancer averages US $22,080 [68], and in the United States, the cost reaches even higher levels of US$32,500 [69]. Lip and oral cavity cancers together represent the 16th most common neoplasm worldwide, with nearly 355,000 new diagnoses and more than 177,000 estimated deaths in 2018 [70]. Even in this context, most cases are treated at advanced stages, leading to worse prognosis, more aggressive treatments and high costs, reinforcing the importance that early detection methods are of great value to the public health system in economic and social terms.

The main perspective for such methods based on autofluorescence and fluorescent probes is that their inclusion can be beneficial when used mainly by general practice dentists (GPD). Recent studies carried out by Simonato et al. [71] and Farah et al. [72], showed that direct visualization had high diagnostic value and improved detection of OPMD during population screening. Current evidence testing such methods in population screening is scarce and still represents a challenge. However, such studies are of fundamental importance and must consider some aspects, as described by Tomo et al. [73]: 1) the type of examiner (GPD or specialist); 2) previously known diagnosis of OPMD; 3) The COE must be performed and the diagnostic values must be presented for the FV and the COE alone and together; 4) Biopsy must be performed to obtain a definitive diagnosis of such lesions; 5) Larger samples must be reached to characterize population screenings.

It is suggested, for future papers in this field, that they include a larger sample, taking into account an 'n' greater than 150, since, in this meta-analysis, the articles with greater robustness, i.e. that produce more reliable results, presented an 'n' greater than 100 [19,22,29]. In addition, the availability of statistical data is of great importance to enable the carrying out of meta-analysis. Clinical aspects can also be considered, such as the implementation of software that can quantify the

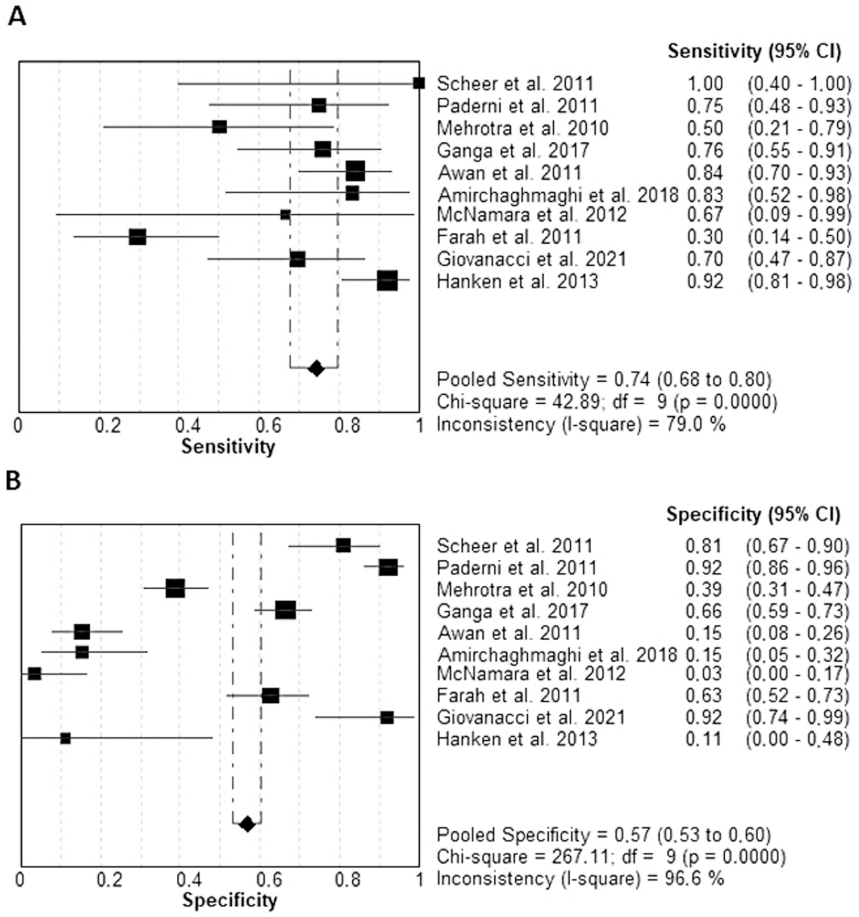

Fig. 2. Plots showing the values obtained for sensitivity and specificity after statistical analysis.

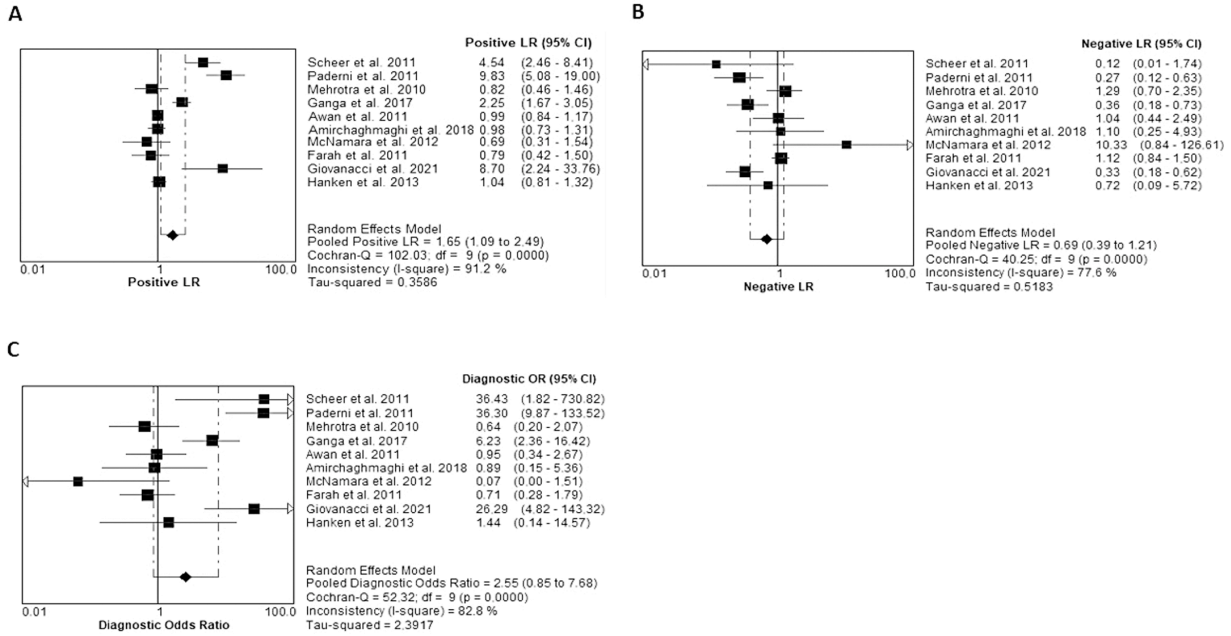

Fig. 3. Plots showing the values obtained for positive likelihood ratio (positive LR), negative likelihood ratio (negative LR) and diagnostic odds ratio (DOR).

autofluorescence, which would allow the standardization of results and their improved interpretation. According Betz et al. [74,75], epithelial lesions that are flat were better delineated compared to large exophytic lesions by autofluorescence, which justifies their use as a screening method for premalignant and malignant oral lesions by dentists.

5. Conclusion

The inclusion of autofluorescence-based methods in the clinical routine is promising and may assist in the early detection of potentially malignant oral diseases. However, they should be used as auxiliary methods, and it is not possible to suggest the replacement of the histopathological exam for diagnosis. In the present meta-analysis, the autofluorescence technique had a pooled sensitivity of 57% and pooled specificity of 74%. It is suggested that further studies be carried out to improve its protocol, as well as to demonstrate the ability to track non-evident lesions on clinical examination and on those patients with a history of injuries, not exposing them to biopsies frequently. Regarding fluorescent probes, from a practical point of view, their use cannot be justified in the clinical routine, as they have a long incubation time period.

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Appendix 1

Studies excluded in the full-text analysis and the reasons for exclusion.

Author, year	Reason for exclusion
Filiurin (1993)	Article in Russian
Sawan; Mashlah (2015)	They do not mention the definitive diagnosis
Kaur; Jacobs (2015)	They do not mention the definitive diagnosis
Laronde et al. (2014)	They do not mention the definitive diagnosis
Tsai et al. (2003)	Non-diagnostic study
de Veld et al. (2004)	Non-diagnostic study
Vigneswaran	Non-diagnostic study
Kleinpenning et al. (2010)	Non-diagnostic study
Jayanthi et al. (2009)	Non-diagnostic study
Kanick et al. (2014)	Non-diagnostic study
Kanchwala et al. (2018)	Non-diagnostic study
Wang et al. (2009)	Non-diagnostic study
Nazeer et al. (2014)	Non-diagnostic study
Patil et al. (2018)	Non-diagnostic study
Arifler et al. (2005)	Non-diagnostic study
Obstoy et al. (2015)	Animals
Skala et al. (2004)	Animals

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4 CONSIDERAÇÕES FINAIS

O uso de sondas fluorescentes e autofluorescência demonstraram importante potencial diante da detecção de desordens orais potencialmente malignas. Por apresentarem diferentes características, o uso de métodos adjuntos pode auxiliar, dentro da rotina clínica, os cirurgiões-dentistas a identificar as mais diferentes lesões em estágios iniciais, permitindo um tratamento menos invasivo e garantindo prognósticos mais favoráveis aos pacientes. Ressalta-se que o uso de tais ferramentas não substitui a biópsia e análise histopatológica; no entanto, pode delimitar, as áreas mais representativas para a realização da mesma, como também pode ser utilizada dentro de estratégias de saúde para o rastreamento e acompanhamento de pacientes classificados de alto risco para o desenvolvimento de tais lesões.

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